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                   frequency
 NEWS  5  Feb 19   Access via Tymnet and SprintNet Eliminated Effective 3/31/02
 NEWS  6  Mar 08   Gene Names now available in BIOSIS
 NEWS  7  Mar 22   TOXLIT no longer available
 NEWS  8  Mar 22   TRCTHERMO no longer available
 NEWS  9  Mar 28   US Provisional Priorities searched with P in CA/CAplus
                   and USPATFULL
 NEWS 10  Mar 28   LIPINSKI/CALC added for property searching in REGISTRY
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 NEWS 16  Apr 22   Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
 NEWS 17  Apr 22   BIOSIS Gene Names now available in TOXCENTER
 NEWS 18  Apr 22   Federal Research in Progress (FEDRIP) now available

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uspatall
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CA INDEXING COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

=> e lubitz werner/au
E1          684     LUBITZ W/AU
E2            4     LUBITZ W */AU
E3          154 --> LUBITZ WERNER/AU
E4          101     LUBITZ WOLFGANG/AU
E5            1     LUBITZKI LOTHAR/AU
E6            5     LUBITZSCH P/AU
E7            7     LUBITZSCH PETER/AU
E8            1     LUBITZSCH R/AU
E9            6     LUBITZSCH W/AU
E10           6     LUBITZSCH WOLFGANG/AU
E11           1     LUBIVYI V G/AU
E12           1     LUBIZ M/AU

=> s e1-e3
L1          842 ("LUBITZ W"/AU OR "LUBITZ W *"/AU OR "LUBITZ WERNER"/AU)

=> e sleytr uwe/au
E1            1     SLEYTR U I/AU
E2            1     SLEYTR UVE B/AU
E3            8 --> SLEYTR UWE/AU
E4          210     SLEYTR UWE B/AU
E5           15     SLEYTR UWE BERND/AU
E6            1     SLEYTR UWE R/AU
E7            1     SLEYTRE B/AU
E8            1     SLEZ AK J/AU
E9            1     SLEZ AK K/AU
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E10          10      SLEZ AK P/AU
E11           1      SLEZ L/AU
E12          13      SLEZ L G/AU

=> s e1-e5
L2          235 ("SLEYTR U I"/AU OR "SLEYTR UVE B"/AU OR "SLEYTR UWE"/AU OR
                "SLEYTR UWE B"/AU OR "SLEYTR UWE BERND"/AU)

=> e sleytr u/au
E1            1      SLEYTR B/AU
E2            1      SLEYTR L/AU
E3           44 --> SLEYTR U/AU
E4          550      SLEYTR U B/AU
E5            1      SLEYTR U I/AU
E6            1      SLEYTR UVE B/AU
E7            8      SLEYTR UWE/AU
E8          210      SLEYTR UWE B/AU
E9           15      SLEYTR UWE BERND/AU
E10           1      SLEYTR UWE R/AU
E11           1      SLEYTRE B/AU
E12           1      SLEZ AK J/AU

=> s e3
L3           44 "SLEYTR U"/AU

=> e kuen beatrix/au
E1            1      KUEN B S/AU
E2            1      KUEN BEA/AU
E3           25 --> KUEN BEATRIX/AU
E4            3      KUEN C/AU
E5            1      KUEN C F/AU
E6            2      KUEN C K/AU
E7            2      KUEN C L/AU
E8            1      KUEN C Y/AU
E9            1      KUEN CHAN/AU
E10           1      KUEN CHAN LAU/AU
E11           1      KUEN CHAN PAK/AU
E12           2      KUEN CHI FOUN/AU

=> s e1-e3
L4           27 ("KUEN B S"/AU OR "KUEN BEA"/AU OR "KUEN BEATRIX"/AU)

=> e kuen b/au
E1            1      KUEN A B/AU
E2            1      KUEN AU WING/AU
E3           48 --> KUEN B/AU
E4            1      KUEN B I/AU
E5            1      KUEN B S/AU
E6            1      KUEN BEA/AU
E7           25      KUEN BEATRIX/AU
E8            3      KUEN C/AU
E9            1      KUEN C F/AU
E10           2      KUEN C K/AU
E11           2      KUEN C L/AU
E12           1      KUEN C Y/AU

=> s e3
L5           48 "KUEN B"/AU

=> e truppe michaela/au
E1            5      TRUPPE MICHAEL/AU
E2            2      TRUPPE MICHAEL J/AU
E3            5 --> TRUPPE MICHAELA/AU
E4            1      TRUPPE MICHAELA SONJA/AU
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E5             2      TRUPPE P/AU
E6             1      TRUPPE PETER/AU
E7             1      TRUPPE R B/AU
E8             1      TRUPPE ROBERT B/AU
E9             1      TRUPPE ROBERT E/AU
E10            2      TRUPPE S/AU
E11           60      TRUPPE W/AU
E12            8      TRUPPE WOLFGANG/AU

=> s e1-e4
L6            13  ("TRUPPE MICHAEL"/AU OR "TRUPPE MICHAEL J"/AU OR "TRUPPE MICHAEL
                  A"/AU OR "TRUPPE MICHAELA SONJA"/AU)

=> e howorka stefan/au
E1             1      HOWORKA S G/AU
E2             1      HOWORKA SIEGFRIED/AU
E3            17  --> HOWORKA STEFAN/AU
E4             1      HOWORKA STEFAN G/AU
E5             2      HOWORKA STEPHAN/AU
E6             1      HOWORKA WILFRIED/AU
E7             1      HOWORKO ADOLF C/AU
E8             1      HOWORKO N/AU
E9             1      HOWORT P/AU
E10           14      HOWORTH A/AU
E11           13      HOWORTH A J/AU
E12            1      HOWORTH ALISON/AU

=> s e3-e5
L7            20  ("HOWORKA STEFAN"/AU OR "HOWORKA STEFAN G"/AU OR "HOWORKA STEPHA
                  N"/AU)

=> e howorka s/au
E1             2      HOWORKA M/AU
E2             2      HOWORKA MATTHIAS/AU
E3            31  --> HOWORKA S/AU
E4             1      HOWORKA S G/AU
E5             1      HOWORKA SIEGFRIED/AU
E6            17      HOWORKA STEFAN/AU
E7             1      HOWORKA STEFAN G/AU
E8             2      HOWORKA STEPHAN/AU
E9             1      HOWORKA WILFRIED/AU
E10            1      HOWORKO ADOLF C/AU
E11            1      HOWORKO N/AU
E12            1      HOWORT P/AU

=> s e3-e8
L8            53  ("HOWORKA S"/AU OR "HOWORKA S G"/AU OR "HOWORKA SIEGFRIED"/AU
                  OR "HOWORKA STEFAN"/AU OR "HOWORKA STEFAN G"/AU OR "HOWORKA
                  STEPHAN"/AU)

=> e resch stephanka/au
E1             1      RESCH STEPANKA/AU
E2             4      RESCH STEPHANIE/AU
E3             0  --> RESCH STEPHANKA/AU
E4             1      RESCH STEPHNIE/AU
E5             1      RESCH STEVEN C/AU
E6             5      RESCH SYLVIA/AU
E7            48      RESCH T/AU
E8             1      RESCH T A/AU
E9             1      RESCH T J/AU
E10            1      RESCH T L/AU
E11            3      RESCH TH/AU
E12            2      RESCH THOMAS/AU
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=> s e1-e2
L9             5 ("RESCH STEPANKA"/AU OR "RESCH STEPHANIE"/AU)

=> s resch s/au
L10           86 RESCH S/AU

=> e resch s/au
E1             1      RESCH RONALD D/AU
E2             1      RESCH RONALD DALE/AU
E3            86 --> RESCH S/AU
E4             5      RESCH S C/AU
E5             1      RESCH S L/AU
E6             6      RESCH SEDLMEIER G/AU
E7             1      RESCH SHANE/AU
E8             2      RESCH SHANE L/AU
E9             6      RESCH STEFAN/AU
E10            1      RESCH STEFANIE/AU
E11            1      RESCH STEPANKA/AU
E12            4      RESCH STEPHANIE/AU

=> s e3
L11           86 "RESCH S"/AU

=> e schroll gerhard/au
E1            14      SCHROLL GENE E/AU
E2             2      SCHROLL GEORG/AU
E3            12 --> SCHROLL GERHARD/AU
E4             2      SCHROLL GOTTFRIED/AU
E5             4      SCHROLL GUENTER/AU
E6            47      SCHROLL GUSTAV/AU
E7            30      SCHROLL H/AU
E8             1      SCHROLL HANS/AU
E9             2      SCHROLL HARSTED BJARNE/AU
E10            2      SCHROLL HENNING/AU
E11            7      SCHROLL J/AU
E12            1      SCHROLL J T/AU

=> s e3
L12           12 "SCHROLL GERHARD"/AU

=> e schroll g/au
E1             1      SCHROLL FRANZ HEINZ/AU
E2             1      SCHROLL FRITZ/AU
E3            43 --> SCHROLL G/AU
E4             1      SCHROLL G E/AU
E5             3      SCHROLL GENE/AU
E6            14      SCHROLL GENE E/AU
E7             2      SCHROLL GEORG/AU
E8            12      SCHROLL GERHARD/AU
E9             2      SCHROLL GOTTFRIED/AU
E10            4      SCHROLL GUENTER/AU
E11           47      SCHROLL GUSTAV/AU
E12           30      SCHROLL H/AU

=> s e3
L13           43 "SCHROLL G"/AU

=> e sara margit/au
E1             9      SARA M N/AU
E2             4      SARA MAAGARETSUTO JIENKINZU/AU
E3           137 --> SARA MARGIT/AU
E4             3      SARA MARTIN N/AU
E5             9      SARA MAURIZIO/AU
E6             1      SARA MAUSHIYU/AU
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E7            1      SARA MEESON RITSUCHIMONDO/AU
E8            1      SARA MESHIERI/AU
E9            1      SARA MEZA C R/AU
E10          39      SARA MICHELE/AU
E11           1      SARA MIRUDORETSUDO BISHIYOTSUPU/AU
E12           1      SARA MOUNA/AU

=> s e1-e3
L14         150 ("SARA M N"/AU OR "SARA MAAGARETSUTO JIENKINZU"/AU OR "SARA
                MARGIT"/AU)

=>

=> e sara m/au
E1            1      SARA LAFOSSE V/AU
E2            2      SARA LASZLO/AU
E3          370 --> SARA M/AU
E4            8      SARA M */AU
E5            1      SARA M I/AU
E6            5      SARA M J/AU
E7            9      SARA M N/AU
E8            4      SARA MAAGARETSUTO JIENKINZU/AU
E9          137      SARA MARGIT/AU
E10           3      SARA MARTIN N/AU
E11           9      SARA MAURIZIO/AU
E12           1      SARA MAUSHIYU/AU

=> s e3-e4
L15         378 ("SARA M"/AU OR "SARA M *"/AU)

=> s l1-l15
L16        1634 (L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR L7 OR L8 OR L9
                OR L10 OR L11 OR L12 OR L2

=> s l16 and s layer protein
   6 FILES SEARCHED...
L17         258 L16 AND S LAYER PROTEIN

=> s  l17 and (mature or complete or crystalline)
L18         118 L17 AND (MATURE OR COMPLETE OR CRYSTALLINE)

=> dup rem l18
PROCESSING COMPLETED FOR L18
L19          43 DUP REM L18 (75 DUPLICATES REMOVED)

=> d bib ab 1-43

L19  ANSWER 1 OF 43     MEDLINE                          DUPLICATE 1
AN   2002200210     IN-PROCESS
DN   21930707   PubMed ID: 11932495
TI   A recombinant bacterial cell surface (S-layer)-major birch pollen
     allergen-fusion protein (rSbsC/Bet v1) maintains the ability to
     self-assemble into regularly structured monomolecular lattices and the
     functionality of the allergen.
AU   Breitwieser Andreas; Egelseer Eva M; Moll Dieter; Ilk Nicola; Hotzy
     Christoph; Bohle Barbara; Ebner Christof; Sleytr Uwe B;
     Sara Margit
CS   Center for Ultrastructure Research and Ludwig Boltzmann-Institute for
     Molecular Nanotechnology, University of Agricultural Sciences, Gregor
     Mendelstrasse 33, 1180 Vienna and Institute of Pathophysiology, University
     of Vienna, 1090 Vienna, Austria.
SO   PROTEIN ENGINEERING, (2002 Mar) 15 (3) 243-9.
     Journal code: 8801484. ISSN: 0269-2139.
CY   England: United Kingdom
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DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020405
Last Updated on STN: 20020405
AB The **mature crystalline** bacterial cell surface (**S-layer) protein** SbsC of Bacillus stearothermophilus ATCC 12980 comprises amino acids 31-1099 and assembles into an oblique lattice type. As the deletion of up to 179 C-terminal amino acids did not interfere with the self-assembly properties of SbsC, the sequence encoding the major birch pollen allergen (Bet v1) was fused to the sequence encoding the truncated form rSbsC(31-920). The S-layer fusion protein, termed rSbsC/Bet v1, maintained the ability to self-assemble into flat sheets and open-ended cylinders. The presence and the functionality of the fused Bet v1 sequence was proved by blot experiments using BIP1, a monoclonal antibody against Bet v1 and Bet v1-specific IgE-containing serum samples from birch pollen allergic patients. The location and accessibility of the allergen moiety on the outer surface of the S-layer lattice were demonstrated by immunogold labeling of the rSbsC/Bet v1 monolayer, which was obtained by oriented recrystallization of the S-layer fusion protein on native cell wall sacculi. Thereby, the specific interactions between the N-terminal part of SbsC and a distinct type of secondary cell wall polymer were exploited. This is the first S-layer fusion protein described that had retained the specific properties of the **S-layer protein** moiety in addition to those of the fused functional peptide sequence.

L19 ANSWER 2 OF 43 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 2002013097 EMBASE
TI Characterization of an S-layer glycoprotein produced in the course of S-layer variation of Bacillus stearothermophilus ATCC 12980 and sequencing and cloning of the sbsD gene encoding the protein moiety.
AU Egelseer E.M.; Danhorn T.; Pleschberger M.; Hotzy C.; Sleytr U.B.; **Sara M.**
CS E.M. Egelseer, Center Ultrastructure Research, Ludwig Boltzmann-Ins. Molec. Nanot., University of Agricultural Sciences, 1180 Vienna, Austria. egelseer@edv1.boku.ac.at
SO Archives of Microbiology, (2002) 177/1 (70-80).
Refs: 39
ISSN: 0302-8933 CODEN: AMICCW
CY Germany
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered by an oblique lattice which consists of the **S-layer protein** SbsC. On SDS-polyacrylamide gels, the **mature S-layer protein** migrates as a single band with an apparent molecular mass of 122 kDa. During cultivation of B. stearothermophilus ATCC 12980 at 67.degree.C instead of 55.degree.C, a variant developed that had a secondary cell wall polymer identical to that of the wild-type strain, but it carried an S-layer glycoprotein that could be separated on SDS-polyacrylamide gels into four bands with apparent molecular masses of 92, 118, 150 and 175 kDa. After deglycosylation, only a single protein band with a molecular mass of 92 kDa remained. The **complete** nucleotide sequence encoding the protein moiety of this S-layer glycoprotein, termed SbsD, was established by PCR and inverse PCR. The sbsD gene of 2,709 bp is predicted to encode a protein of 96.2 kDa with a 30-amino-acid signal peptide. Within the 807 bp encoding the signal peptide and the N-terminal sequence (amino acids 31-269), different nucleotides for sbsD and sbsC were observed in 46 positions, but 70% of these mutations were silent, thus leading to a level of identity of 95% for the N-terminal parts. The level of identity of the

remaining parts of SbsD and SbsC was below 10%, indicating that the lysine-, tyrosine- and arginine-rich N-terminal region in combination with a distinct type of secondary cell wall polymer remained conserved upon S-layer variation. The sbsD sequence encoding the **mature** **S-layer protein** cloned into the pET28a vector led to stable expression in Escherichia coli HMS174(DE3). This is the first example demonstrating that S-layer variation leads to the synthesis of an S-layer glycoprotein.

L19 ANSWER 3 OF 43 USPATFULL
AN 2001:167594 USPATFULL
TI Method of producing a structured layer
IN **Sleytr, Uwe B.**, Vienna, Austria
Pum, Dietmar, Vienna, Austria
Loschner, Hans, Vienna, Austria
PA IMS-Ionen Mikrofabrikations Systems GmbH, Vienna, Austria (non-U.S. corporation)
PI US 6296700 B1 20011002
AI US 1999-388289 19990901 (9)
RLI Continuation-in-part of Ser. No. WO 1998-AT50, filed on 4 Mar 1998
PRAI AT 1997-373 19970304
DT Utility
FS GRANTED
EXNAM Primary Examiner: Kunemund, Robert
LREP Vigil, Thomas R.
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 15 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 387
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to a method for producing a structured layer of defined functional molecules on the surface of a flat substrate, on the surface of which structures having different surface properties, at least as regards their hydrophobicity, are produced. A monolayer of a protein-containing **crystalline** cell surface layer (S layer) is deposited by recrystallization on said structured surface. Said S Layer binds only to those structured areas of the surface characterized by raised hydrophobicity. Alternatively, a structured S-layer may also be produced on the basis of a monolayer of an S layer deposited on a substrate by irradiating predefined sections of said layer to be structured with radiation of a predetermined intensity and energy. In the irradiated sections of the S-layer this suppresses the binding or intercalating ability of at least one surface. Functional molecules can bind to or become intercalated in the surface or the intermediate spaces of the crystal lattice, for example for use as bio-sensors.

L19 ANSWER 4 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2001:289673 BIOSIS
DN PREV200100289673
TI Analysis of the structure-function relationship of the **S-layer protein** SbsC of Bacillus stearothermophilus ATCC 12980 by producing truncated forms.
AU Jarosch, Marina; Egelseer, Eva M.; Huber, Carina; Moll, Dieter; Mattanovich, Diethard; **Sleytr, Uwe B.**; **Sara, Margit (1)**
CS (1) Centre for Ultrastructure Research, Ludwig Boltzmann-Institute for Molecular Nanotechnology, University of Agricultural Sciences, 1180, Vienna: sara@edv1.boku.ac.at Austria
SO Microbiology (Reading), (May, 2001) Vol. 147, No. 5, pp. 1353-1363. print. ISSN: 1350-0872.
DT Article
LA English
SL English
AB The **mature** surface layer (**S-layer**)

protein SbsC of Bacillus stearothermophilus ATCC 12980 comprises amino acids 31-1099 and self-assembles into an oblique lattice type which functions as an adhesion site for a cell-associated high-molecular-mass exoamylase. To elucidate the structure-function relationship of distinct segments of SbsC, three N- and seven C-terminal truncations were produced in a heterologous expression system, isolated, purified and their properties compared with those of the recombinant **mature** **S-layer protein** rSbsC31-1099. With the various truncated forms it could be demonstrated that the N-terminal part (aa 31-257) is responsible for anchoring the S-layer subunits via a distinct type of secondary cell wall polymer to the rigid cell wall layer, but this positively charged segment is not required for the self-assembly of SbsC, nor for generating the oblique lattice structure. If present, the N-terminal part leads to the formation of in vitro double-layer self-assembly products. Affinity studies further showed that the N-terminal part includes an exoamylase-binding site. Interestingly, the N-terminal part carries two sequences of 6 and 7 aa (AKAALD and KAAYEAA) that were also identified on the amylase-binding protein AbpA of Streptococcus gordonii. In contrast to the self-assembling N-terminal truncation rSbsC258-1099, two further N-terminal truncations (rSbsC343-1099, rSbsC447-1099) and three C-terminal truncations (rSbsC31-713, rSbsC31-844, rSbsC31-860) had lost the ability to self-assemble and stayed in the water-soluble state. Studies with the self-assembling C-terminal truncations rSbsC31-880, rSbsC31-900 and rSbsC31-920 revealed that the C-terminal 219 aa can be deleted without interfering with the self-assembly process, while the C-terminal 179 aa are not required for the formation of the oblique lattice structure.

L19 ANSWER 5 OF 43 MEDLINE DUPLICATE 3
AN 2002053459 IN-PROCESS
DN 21637203 PubMed ID: 11778930
TI Development of affinity microparticles for extracorporeal blood purification based on **crystalline** bacterial cell surface proteins.
AU Weber V; Weigert S; **Sara M**; Sleytr U B; Falkenhagen D
CS Christian Doppler Laboratory for Specific Adsorption Technologies in Medicine, Centre for Biomedical Technology, Donau-University Krems, Austria.. weber@donau-uni.ac.at
SO THERAPEUTIC APHERESIS, (2001 Oct) 5 (5) 433-8.
Journal code: 9706703. ISSN: 1091-6660.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020125
Last Updated on STN: 20020125
AB In this article, the development of specific adsorbents for extracorporeal blood purification are described. Affinity microparticles were prepared by linking Protein A to **crystalline** cell surface layers (S-layers) from Thermoanaerobacter thermohydrosulfuricus 1111-69. S-layers were used in the form of cell wall fragments obtained by breaking whole cells by ultrasonification, resulting in cup-shaped structures (average size 0.5 x 1 microm) completely covered with **S-layer** **protein**. Protein A was covalently bound to carboxylic acid groups of the **S-layer protein** after activation with 1-ethyl-3,3'(dimethylamino)propylcarbodiimide. In batch adsorption experiments with fresh frozen human plasma, the resulting S-layer based affinity microparticles showed a high adsorption capacity for IgG (40 mg IgG were bound per g wet pellet of S-layer based affinity microparticles). Fractions eluted from the microparticles were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. They contained only IgG demonstrating that adsorption was specific. In biocompatibility tests, preparations of the S-layer microparticles showed no low-density lipoprotein-reactivity, no cytotoxicity, and no cytokine inducing

activity.

L19 ANSWER 6 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2002:146741 BIOSIS
DN PREV200200146741
TI Characterization of an S-layer glycoprotein produced in the course of S-layer variation of Bacillus stearothermophilus ATCC 12980 and sequencing and cloning of the sbsD gene encoding the protein moiety.
AU Egelseer, Eva M. (1); Danhorn, Thomas; Pleschberger, Magdalena; Hotzy, Christoph; **Sleytr, Uwe B.**; **Sara, Margit**
CS (1) Center for Ultrastructure Research, Ludwig Boltzmann-Institute for Molecular Nanotechnology, University of Agricultural Sciences, 1180, Vienna: egelseer@edv1.boku.ac.at Austria
SO Archives of Microbiology, (December, 2001) Vol. 177, No. 1, pp. 70-80. http://www.link.springer.de/link/service/journals/00203/index.htm. print. ISSN: 0302-8933.
DT Article
LA English
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered by an oblique lattice which consists of the **S-layer protein** SbsC. On SDS-polyacrylamide gels, the **mature S-layer protein** migrates as a single band with an apparent molecular mass of 122 kDa. During cultivation of B. stearothermophilus ATCC 12980 at 67degreeC instead of 55degreeC, a variant developed that had a secondary cell wall polymer identical to that of the wild-type strain, but it carried an S-layer glycoprotein that could be separated on SDS-polyacrylamide gels into four bands with apparent molecular masses of 92, 118, 150 and 175 kDa. After deglycosylation, only a single protein band with a molecular mass of 92 kDa remained. The **complete** nucleotide sequence encoding the protein moiety of this S-layer glycoprotein, termed SbsD, was established by PCR and inverse PCR. The sbsD gene of 2,709 bp is predicted to encode a protein of 96.2 kDa with a 30-amino-acid signal peptide. Within the 807 bp encoding the signal peptide and the N-terminal sequence (amino acids 31-269), different nucleotides for sbsD and sbsC were observed in 46 positions, but 70% of these mutations were silent, thus leading to a level of identity of 95% for the N-terminal parts. The level of identity of the remaining parts of SbsD and SbsC was below 10%, indicating that the lysine-, tyrosine- and arginine-rich N-terminal region in combination with a distinct type of secondary cell wall polymer remained conserved upon S-layer variation. The sbsD sequence encoding the **mature S-layer protein** cloned into the pET28a vector led to stable expression in Escherichia coli HMS174(DE3). This is the first example demonstrating that S-layer variation leads to the synthesis of an S-layer glycoprotein.

L19 ANSWER 7 OF 43 CAPLUS COPYRIGHT 2002 ACS
AN 2001:593499 CAPLUS
DN 135:300796
TI Conserved anchoring mechanisms between **crystalline** cell surface S-layer proteins and secondary cell wall polymers in Gram-positive bacteria?
AU **Sara, Margit**
CS Institute for Molecular Nanotechnology, Ctr. for Ultrastructure Research and Ludwig Boltzmann, University of Agricultural Sciences, Vienna, 1180, Austria
SO Trends in Microbiology (2001), 9(2), 47-49
CODEN: TRMIEA; ISSN: 0966-842X
PB Elsevier Science Ltd.
DT Journal
LA English
AB In Gram-pos. bacteria, the primary function of the rigid cell wall layer which comprises peptidoglycan and accessory cell wall polymers is to provide an exoskeleton for protection against mech. and osmotic stress and

serve as an attachment site for other proteins interacting with the bacterial environment. S-layer proteins from Gram-pos. bacteria are found via their amino-terminal region to secondary cell wall polymers that are covalently linked to the peptidoglycan backbone. **Complete** structural anal. of selected secondary cell wall polymers showed they are teichuronic acids. S-layer homologous (SLH) motifs are specifically targeted to pyruvylated secondary cell wall polymers. The data obtained using S-layer proteins from Bacillus stearothermophilus strains devoid of SLH motifs and secondary cell wall polymers lacking pyruvic acid residues strongly show the existence of further conserved cell-wall-anchoring mechanisms. The study of Mesnage et al. is also discussed. It reports that, in Bacillus anthracis, pyruvylation of a peptidoglycan-assocd. cell wall polymer is necessary for the attachment of the S-layer proteins EA1 and Sap to the bacterial cell surface.

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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 8 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5

AN 2001:69959 BIOSIS

DN PREV200100069959

TI Surface-accessible residues in the monomeric and assembled forms of a bacterial surface layer protein.

AU **Howorka, Stefan (1)**; **Sara, Margit**; Wang, Yunjuan; **Kuen, Beatrix**; **Sleytr, Uwe B.**; **Lubitz, Werner**; Bayley, Hagan

CS (1) Dept. of Medical Biochemistry and Genetics, The Texas A and M University System Health Science Center, 4440 Reynolds Medical Bldg., College Station, TX, 77843-1114: howorka@medicine.tamu.edu USA

SO Journal of Biological Chemistry, (December 1, 2000) Vol. 275, No. 48, pp. 37876-37886. print.
ISSN: 0021-9258.

DT Article

LA English

SL English

AB The **S-layer protein** SbsB of the thermophilic, Gram-positive organism Bacillus stearothermophilus PV72/p2 forms a **crystalline**, porous array constituting the outermost component of the cell envelope. SbsB has a molecular mass of 98 kDa, and the corresponding S-layer exhibits an oblique lattice symmetry. To investigate the molecular structure and assembly of SbsB, we replaced 75 residues (mainly serine, threonine, and alanine), located throughout the primary sequence, with cysteine, which is not found in the wild-type protein. As determined by electron microscopy, 72 out of 75 mutants formed regularly-structured self-assembly products identical to wild-type, thereby proving that the replacement of most of the selected amino acids by cysteine does not dramatically alter the structure of the protein. The three defective mutants, which showed a greatly reduced ability to self-assemble, were, however, successfully incorporated into S-layer of wild-type protein. Monomeric SbsB mutants and SbsB mutants assembled into S-layers were subjected to a surface accessibility screen by targeted chemical modification with a 5-kDa hydrophilic cysteine-reactive polyethylene glycol conjugate. In the monomeric form of SbsB, 34 of the examined residues were not surface accessible, while 23 were classified as very accessible, and 18 were of intermediate surface accessibility. By contrast, in the assembled S-layers, 57 of the mutated residues were not accessible, six were very accessible, and 12 of intermediate accessibility. Together with other structural information, the results suggest a model for SbsB in which functional domains are segregated along the length of the polypeptide chain.

L19 ANSWER 9 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6

AN 2000:112748 BIOSIS

DN PREV200000112748
TI S-layer-coated liposomes as a versatile system for entrapping and binding target molecules.
AU Mader, Christoph; Kuepcue, Seta; **Sleytr, Uwe B.**; **Sara, Margit (1)**
CS (1) Zentrum fuer Ultrastrukturforschung und Ludwig Boltzmann-Institut fuer Molekulare Nanotechnologie, Universitaet fuer Bodenkultur Wien, Gregor-Mendelstr. 33, A-1180, Vienna Austria
SO Biochimica et Biophysica Acta, (Jan. 15, 2000) Vol. 1463, No. 1, pp. 142-150.
ISSN: 0006-3002.
DT Article
LA English
SL English
AB In the present study, unilamellar liposomes coated with the **crystalline** bacterial cell surface layer (**S-layer**) **protein** of Bacillus stearothermophilus PV72/p2 were used as matrix for defined binding of functional molecules via the avidin- or streptavidin-biotin bridge. The liposomes were composed of dipalmitoyl phosphatidylcholine, cholesterol and hexadecylamine in a molar ratio of 10:5:4 and they had an average size of 180 nm. For introducing specific functions into the S-layer lattice without affecting substances encapsulated within the liposomes, crosslinking and activation reagents had to be identified which did not penetrate the liposomal membrane. Among different reagents, a hydrophilic dialdehyde generated by periodate cleavage of raffinose and a sulfo-succinimide activated dicarboxylic acid were found to be impermeable for the liposomal membrane. Both reagents completely crosslinked the S-layer lattice without interfering with its regular structure. Biotinylation of S-layer-coated liposomes was achieved by coupling p-diazobenzoyl biocytin which preferably reacts with the phenolic residue of tyrosine or with the imidazole ring of histidine. By applying this method, two biotin residues accessible for subsequent avidin binding were introduced per S-layer subunit. As visualized by labeling with biotinylated ferritin, an ordered monomolecular layer of streptavidin was formed on the surface of the S-layer-coated liposomes. As a second model system, biotinylated anti-human IgG was attached via the streptavidin bridge to the biotinylated S-layer-coated liposomes. The biological activity of the bound anti-human IgG was confirmed by ELISA.

L19 ANSWER 10 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:482406 BIOSIS
DN PREV199900482406
TI The effect of **S-layer protein** adsorption and crystallization on the collective motion of a planar lipid bilayer studied by dynamic light scattering.
AU Hirn, Rainer; Schuster, Bernhard; **Sleytr, Uwe B.**; Bayerl, Thomas M. (1)
CS (1) Universitaet Wuerzburg, Physikalisches Institut EP-5, Am Hubland, D-97074, Wuerzburg Germany
SO Biophysical Journal, (Oct., 1999) Vol. 77, No. 4, pp. 2066-2074.
ISSN: 0006-3495.
DT Article
LA English
SL English
AB A dedicated dynamic light scattering (DLS) setup was employed to study the undulations of freely suspended planar lipid bilayers, the so-called black lipid membranes (BLM), over a previously inaccessible spread of frequencies (relaxation times ranging from 10-2 to 10-6 s) and wavevectors (250 cm-1 < q < 38,000 cm-1). For a BLM consisting of 1,2-dielaidoyl-sn-3-glycero-phosphocholine (DEPC) doped with two different proportions of the cationic lipid analog dioctadecyl-dimethylammonium bromide (DODAB) we observed an increase of the lateral tension of the membrane with the DODAB concentration. The experimentally determined dispersion behavior of the transverse shear mode was in excellent agreement with the theoretical

predictions of a first-order hydrodynamic theory. The symmetric adsorption of the **crystalline** bacterial cell surface layer (S-layer) proteins from Bacillus coagulans E38-66 to a weakly cationic BLM (1.5 mol % DODAB) causes a drastic reduction of the membrane tension well beyond the previous DODAB-induced tension increase. The likely reason for this behavior is an increase of molecular order along the lipid chains by the protein and/or partial protein penetration into the lipid headgroup region. **S-layer protein** adsorption to a highly cationic BLM (14 mol % DODAB) shows after 7 h incubation time an even stronger decrease of the membrane tension by a factor of five, but additionally a significant increase of the (previously negligible) surface viscosity, again in excellent agreement with the hydrodynamic theory. Further incubation (24 h) shows a drastic increase of the membrane bending energy by three orders of magnitude as a result of a large-scale, two-dimensional recrystallization of the S-layer proteins at both sides of the BLM. The results demonstrate the potential of the method for the assessment of the different stages of protein adsorption and recrystallization at a membrane surface by measurements of the collective membrane modes and their analysis in terms of a hydrodynamic theory.

L19 ANSWER 11 OF 43 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN 1999-07020 BIOTECHDS
TI **Crystalline** bacterial cell surface layers (S layers): from supramolecular cell structure to biomimetics and nanotechnology; evaluation of the production, characteristics and applications of self-assembling **S-layer protein** membranes; a review
AU Sleytr U B; Messner P; Pum D; **Sara M**
CS Univ.Bodenkultur
LO Zentrum fuer Ultrastrukturforschung und Ludwig-Boltzmann-Institut fuer Molekulare Nanotechnologie, Universitaet fuer Bodenkultur, Gregor-Mendel-Strasse 33 A-1180 Vienna, Austria.
Email: sleytr@edv1.boku.ac.at
SO Angew.Chem.Int.Ed.Engl.; (1999) 38, 8, 1035-54
CODEN: ACIEAY ISSN: 0570-0833
DT Journal
LA English
AB The structure and uses of the **crystalline** cell surface layers (S-layers) of bacteria were reviewed. The review examined the occurrence and ultrastructure of the layers, as well as their assembly and morphogenesis, with particular regard to their self-assembly in vitro, and in vivo. The review also evaluated the chemical composition of S-layers, and their biosynthesis, as well as their molecular biology, and genetics. The review then described their functional aspects and went on to describe their applications. This looked in detail into their applications in biotechnology and biomimetics, especially the use of S-layers as isoporous ultrafiltration membranes, as matrixes for functional molecule immobilization, in vaccine development, and as supporting structures for functional lipid membranes. The review also analyzed the use of S-layers in nanotechnology, with particular regard to recrystallized S-layers on solid supports, the patterning of those layers, and the use of those layers in the production of regularly arranged nanoparticles. The review concluded S-layers represent the most simple self-assembling membranes with great potential in biotechnology. (228 ref)

L19 ANSWER 12 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
AN 1999:173092 BIOSIS
DN PREV199900173092
TI Self-assembly product formation of the Bacillus stearothermophilus PV72/p6 **S-layer protein** SbsA in the course of autolysis of Bacillus subtilis.
AU **Howorka, Stefan; Sara, Margit; Lubitz, Werner**

; **Kuen, Beatrix (1)**
CS (1) Institut fur Mikrobiologie and Genetik, Universitat Wien, Dr. Bohrgasse 9, A-1030, Vienna Austria
SO FEMS Microbiology Letters, (March 15, 1999) Vol. 172, No. 2, pp. 187-196.
ISSN: 0378-1097.
DT Article
LA English
AB In order to achieve high level expression and to study the release of a protein capable of self-assembly, the gene encoding the **crystalline** cell surface (**S-layer**) **protein** SbsA of Bacillus stearothermophilus PV72/p6, including its signal sequence, was cloned and expressed in Bacillus subtilis. To obtain high level expression, a tightly regulated, xylose-inducible, stably replicating multicopy-plasmid vector was constructed. After induction of expression, the **S-layer protein** made up about 15% of the total cellular protein content, which was comparable to the SbsA content of B. stearothermophilus PV72/p6 cells. During all growth stages, SbsA was poorly secreted to the ambient cellular environment by B. subtilis. Extraction of whole cells with guanidine hydrochloride showed that in late stationary growth phase cells 65% of the synthesised SbsA was retained in the peptidoglycan-containing layer, indicating that the rigid cell wall layer was a barrier for efficient SbsA secretion. Electron microscopic investigation revealed that SbsA release from the peptidoglycan-containing layer started in the late stationary growth phase at distinct sites at the cell surface leading to the formation of extracellular self-assembly products which did not adhere to the cell wall surface. In addition, intracellular sheet-like SbsA self-assembly products which followed the curvature of the cell became visible in partly lysed cells. Intracellularly formed self-assembly products remained intact even after **complete** lysis of the rigid cell envelope layer.

L19 ANSWER 13 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8
AN 1999:237343 BIOSIS
DN PREV199900237343
TI Stabilizing effect of an S-layer on liposomes towards thermal or mechanical stress.
AU Mader, C.; Kuepcue, S.; **Sara, M.**; Sleytr, U. B. (1)
CS (1) Zentrum fuer Ultrastrukturforschung und Ludwig Boltzmann-Institut fuer Molekulare Nanotechnologie, Universitaet fuer Bodenkultur Wien, Gregor-Mendelstr. 33, A-1180, Vienna Austria
SO Biochimica et Biophysica Acta, (April 14, 1999) Vol. 1418, No. 1, pp. 106-116.
ISSN: 0006-3002.
DT Article
LA English
SL English
AB Isolated subunits of the **crystalline** cell surface layer (**S-layer**) **protein** of Bacillus stearothermophilus PV72/p2 were recrystallized on positively charged unilamellar liposomes. Liposomes were composed of dipalmitoylphosphatidylcholine (DPPC), cholesterol and hexadecylamine (HDA) in a molar ratio of 10:5:4 and they were prepared by the dehydration-rehydration method followed by an extrusion procedure. The **S-layer protein** to DPPC ratio was 5.7 nmol/mumol which approximately corresponds to the theoretical value estimated by using the areas occupied by the S-layer lattice and the lipid membrane. Coating of the positively charged liposomes with **S-layer protein** resulted in inversion of the zeta-potential from +29.1 mV to -27.1 mV. Covalent crosslinking of the recrystallized **S-layer protein** was achieved with glutaraldehyde. Chemical analysis revealed that almost all amino groups (> 95%) from HDA in the liposomal membrane were involved in the reaction. To study the influence of an S-layer lattice on the stability of

the liposomes, the hydrophilic marker carboxyfluoresceine (CF) was encapsulated and its release was determined for plain and S-layer-coated liposomes in the course of mechanical and thermal challenges. In comparison to plain liposomes, S-layer-coated liposomes released only half the amount of enclosed CF upon exposure to shear forces or ultrasonication as mechanical stress factors. Furthermore, temperature shifts from 25degreeC to 55degreeC and vice versa induced considerably less CF release from S-layer-coated than from plain liposomes. A similar stabilizing effect of the S-layer lattice was observed after glutaraldehyde treatment of plain and S-layer-coated liposomes.

L19 ANSWER 14 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 9
AN 1999:72167 BIOSIS
DN PREV199900072167
TI Identification of two binding domains, one for peptidoglycan and another for a secondary cell wall polymer, on the N-terminal part of the **S-layer protein** SbsB from Bacillus stearothermophilus PV72/p2.
AU **Sara, Margit** (1); Egelseer, Eva M.; Dekitsch, Christine; **Sleytr, Uwe B.**
CS (1) Zentrum Ultrastrukturforschung, Univ. Bodenkultur, Gregor-Mendelstr. 33, 1180 Vienna Austria
SO Journal of Bacteriology, (Dec., 1998) Vol. 180, No. 24, pp. 6780-6783. ISSN: 0021-9193.
DT Article
LA English
AB First studies on the structure-function relationship of the **S-layer protein** from B. stearothermophilus PV72/p2 revealed the coexistence of two binding domains on its N-terminal part, one for peptidoglycan and another for a secondary cell wall polymer (SCWP). The peptidoglycan binding domain is located between amino acids 1 to 138 of the **mature S-layer protein** comprising a typical S-layer homologous domain. The SCWP binding domain lies between amino acids 240 to 331 and possesses a high serine plus glycine content.

L19 ANSWER 15 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 10
AN 1998:428832 BIOSIS
DN PREV199800428832
TI Influence of the secondary cell wall polymer on the reassembly, recrystallization, and stability properties of the **S-layer protein** from Bacillus stearothermophilus PV72/p2.
AU **Sara, Margit** (1); Dekitsch, Christine; Mayer, Harald F.; Egelseer, Eva M.; **Sleytr, Uwe B.**
CS (1) Zent. Ultrastrukturforschung, Univ. Bodenkultur, Gregor-Mendelstr. 33, 1180 Vienna Austria
SO Journal of Bacteriology, (Aug., 1998) Vol. 180, No. 16, pp. 4146-4153. ISSN: 0021-9193.
DT Article
LA English
AB The high-molecular-weight secondary cell wall polymer (SCWP) from Bacillus stearothermophilus PV72/p2 is mainly composed of N-acetylglucosamine (GlcNAc) and N-acetylmannosamine (ManNAc) and is involved in anchoring the **S-layer protein** via its N-terminal region to the rigid cell wall layer. In addition to this binding function, the SCWP was found to inhibit the formation of self-assembly products during dialysis of the guanidine hydrochloride (GHCl)-extracted **S-layer protein**. The degree of assembly (DA; percent assembled from total **S-layer protein**) that could be achieved strongly depended on the amount of SCWP added to the GHCl-extracted **S-layer protein** and decreased from 90 to 10% when the concentration of the SCWP was increased from 10 to

120 mug/mg of **S-layer protein**. The SCWP kept the **S-layer protein** in the water-soluble state and favored its recrystallization on solid supports such as poly-L-lysine-coated electron microscopy grids. Derived from the orientation of the base vectors of the oblique S-layer lattice, the subunits had bound with their charge-neutral outer face, leaving the N-terminal region with the polymer binding domain exposed to the ambient environment. From cell wall fragments about half of the **S-layer protein** could be extracted with 1 M GlcNAc, indicating that the linkage type between the **S-layer protein** and the SCWP could be related to that of the lectin-polysaccharide type. Interestingly, GlcNAc had an effect on the in vitro self-assembly and recrystallization properties of the **S-layer protein** that was similar to that of the isolated SCWP. The SCWP generally enhanced the stability of the **S-layer protein** against endoproteinase Glu-C attack and specifically protected a potential cleavage site in position 138 of the **mature S-layer protein**.

L19 ANSWER 16 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 11
AN 1998:217628 BIOSIS
DN PREV199800217628
TI The S-layer proteins of two Bacillus stearothermophilus wild-type strains are bound via their N-terminal region to a secondary cell wall polymer of identical chemical composition.
AU Egelseer, Eva Maria; Leitner, Karl; Jarosch, Marina; Hotzy, Christoph; Zayni, Sonja; **Sleytr, Uwe B.**; **Sara, Margit (1)**
CS (1) Zentrum fuer Ultrastrukturforschung, Universitaet fur Bodenkultur, Gregor-Mendelstr. 33, 1180 Wien Austria
SO Journal of Bacteriology, (March, 1998) Vol. 180, No. 6, pp. 1488-1495. ISSN: 0021-9193.
DT Article
LA English
AB Two Bacillus stearothermophilus wild-type strains were investigated regarding a common recognition and binding mechanism between the **S-layer protein** and the underlying cell envelope layer. The **S-layer protein** from B. stearothermophilus PV72/p6 has a molecular weight of 130,000 and assembles into a hexagonally ordered lattice. The S-layer from B. stearothermophilus ATCC 12980 shows oblique lattice symmetry and is composed of subunits with a molecular weight of 122,000. Immunoblotting, peptide mapping, N-terminal sequencing of the whole **S-layer protein** from B. stearothermophilus ATCC 12980 and of proteolytic cleavage fragments, and comparison with the **S-layer protein** from B. stearothermophilus PV72/p6 revealed that the two S-layer proteins have identical N-terminal regions but no other extended structurally homologous domains. In contrast to the heterogeneity observed for the S-layer proteins, the secondary cell wall polymer isolated from peptidoglycan-containing sacculi of the different strains showed identical chemical compositions and comparable molecular weights. The S-layer proteins could bind and recrystallize into the appropriate lattice type on native peptidoglycan-containing sacculi from both organisms but not on those extracted with hydrofluoric acid, leading to peptidoglycan of the A1gamma chemotype. Affinity studies showed that only proteolytic cleavage fragments possessing the **complete** N terminus of the **mature** S-layer proteins recognized native peptidoglycan-containing sacculi as binding sites or could associate with the isolated secondary cell wall polymer, while proteolytic cleavage fragments missing the N-terminal region remained unbound. From the results obtained in this study, it can be concluded that S-layer proteins from B. stearothermophilus wild-type strains possess an identical N-terminal region which is responsible for anchoring the S-layer subunits to a secondary cell wall polymer of identical chemical composition.

L19 ANSWER 17 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 12
AN 1998:434363 BIOSIS
DN PREV199800434363
TI A novel dipstick developed for rapid Bet v 1-specific IgE detection: Recombinant allergen immobilized via a monoclonal antibody to **crystalline** bacterial cell-surface layers.
AU Breitwieser, A. (1); Mader, C. (1); Schocher, I. (1); Hoffmann-Sommergruber, K.; Aberer, W.; Scheiner, O.; Sleytr, U. B.; **Sara, M.** **(1)**
CS (1) Zentrum fuer Ultrastrukturforschung, Univ Bodenkultur, Gregor-Mendelstr. 33, 1180 Wien Austria
SO Allergy (Copenhagen), (Aug., 1998) Vol. 53, No. 8, pp. 786-793. ISSN: 0105-4538.
DT Article
LA English
AB The incidence of allergy to airborne proteins derived from tree and grass pollen, feces of mites, spores of molds, and pet dander has been increasing over the last decades. Since precise diagnosis is a prerequisite for successful immunotherapy, there is a rising demand for rapid, reliable, and inexpensive screening methods such as dipstick assays. With the purified recombinant major birch-pollen allergen rBet v 1a as model protein, **crystalline** bacterial cell-surface layers (S-layers) were tested for their applicability as an immobilization matrix for dipstick development. For this purpose, S-layers were deposited on a mechanically stable microporous support, cross-linked with glutaraldehyde, and free carboxylic acid groups of the **S-layer protein** were activated with carbodiimide. In the present test system, rBet v 1a was immobilized via the monoclonal mouse antibody BIP 1, which, unlike the allergen, is too large to enter the pores of the S-layer lattice, and which therefore formed a closed monolayer on the outermost surface of the crystal lattice. Moreover, BIP 1 is known to modulate IgE binding to the allergen. After incubation of the dipsticks in serum, washing of the reaction zone under tap water, and binding of an anti-IgE alkaline phosphatase conjugate, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium was used as substrate, forming an IgE concentration-dependent colored precipitate on the S-layer surface. The investigation of patient sera previously tested with the CAP system confirmed the specificity of the S-layer-based dipstick assay. Since the dipstick is easy to handle and the whole test procedure takes only 90 min, this test system should be applicable for rapid determination of specific IgE and for first screening in the doctor's practice.

L19 ANSWER 18 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:87452 BIOSIS
DN PREV199800087452
TI Bet v 1, the major birch pollen allergen, conjugated to **crystalline** bacterial cell surface proteins, expands allergen-specific T cells of the Th1/Th0 phenotype in vitro by induction of IL-12.
AU Jahn-Schmid, Beatrice; Siemann, Ute; Zenker, Andrea; Bohle, Barbara; Messner, Paul; Unger, Frank M.; **Sleytr, Uwe B.**; Scheiner, Otto; Kraft, Dietrich; Ebner, Christof (1)
CS (1) Inst. Allgemeine Experimentelle Pathologie, Univ. Wien, AKH-EWB-OST 3Q, Waehringer Guertel 18-20, 1090 Wien Austria
SO International Immunology, (Dec., 1997) Vol. 9, No. 12, pp. 1867-1874. ISSN: 0953-8178.
DT Article
LA English
AB Modulation of allergic immune responses by using adequate adjuvants is a promising concept for future immunotherapy of type I hypersensitivity. In the present study, recombinant Bet v 1 (rBet v 1, the major birch pollen

allergen) was conjugated to cross-linked **crystalline** surface layer proteins (SL) derived from Gram-positive eubacteria. T cell lines (TCL) and clones (TCC) were established from peripheral blood of birch pollen-allergic patients. TCL and TCC were induced either using rBet v 1 alone or rBet v 1/SL conjugates (rBet v 1/SL) as initial antigen stimulus. Cytokine production after re-stimulation with rBet v 1 was investigated. TCL initiated with rBet v 1/SL showed significantly increased IFN-gamma production as compared to rBet v 1-selected TCL. TCC were established from TCL of five patients. As expected, the majority of CD4+ TCC induced by rBet v 1 (55%) displayed a Th2-like pattern of cytokine production. However, only 21% of Bet v 1 -specific TCC isolated from TCL established with the Bet v 1/SL revealed this phenotype. The majority of SL-specific TCC (80%) belonged to the Th1 phenotype. In cultures of peripheral blood mononuclear cells, both, SL and Bet v 1/SL (but not rBet v 1) stimulated the production of high levels of IL-12, a pivotal mediator of Th1 responses. Moreover, stimulation of rBet v 1-induced TCC with rBet v 1/SL led to an Increased IFN-gamma production. This effect could be reversed by neutralizing anti-IL-12 mAb. Together these results indicate an adjuvant effect of SL mediated by IL-12. Our results indicate that bacterial components, such as SL, displaying adjuvant effects may be suitable for immunotherapeutical vaccines for type I allergy.

L19 ANSWER 19 OF 43 CAPLUS COPYRIGHT 2002 ACS
AN 1997:750056 CAPLUS
DN 127:356678
TI Physico-chemical properties of **crystalline** nanoscale enzyme-protein-metal layer composites in biosensors
AU Pum, Dietmar; Neubauer, Angela; **Sleytr, Uwe B.**; Pentzien, Simone; Reetz, Susanne; Kautek, Wolfgang
CS Ludwig Boltzmann Inst. Molecular Nanotechnology, Universitat Bodenkultur Wien, Vienna, A-1180, Austria
SO Ber. Bunsen-Ges. (1997), 101(11), 1686-1689
CODEN: BBPCAX; ISSN: 0940-483X
PB Wiley-VCH Verlag GmbH
DT Journal
LA English
AB Cryst. bacterial cell surface proteins (S-layers) are introduced as immobilization matrixes for binding biol. functional mols. in the development of amperometric biosensors. Due to the cryst. character of S-layers, functional groups are located in well defined position and orientation on the protein meshwork and thus allow to bind mols. in densely packed monolayers. One of the most crit. procedures in the development of amperometric biosensors is the deposition of an elec. contact onto the sensing layer. Conventional sputtering techniques using Pt or Au, or, more recently, pulsed laser deposition of precious metals under inert gas conditions allow to establish elec. conductive contacts on S-layer-based enzyme electrodes.

L19 ANSWER 20 OF 43 MEDLINE DUPLICATE 13
AN 97422863 MEDLINE
DN 97422863 PubMed ID: 9276928
TI Molecular biology of S-layers.
AU Bahl H; Scholz H; Bayan N; Chami M; Leblon G; Gulik-Krzywicki T; Shechter E; Fouet A; Mesnage S; Tosi-Couture E; Gounon P; Mock M; Conway de Macario E; Macario A J; Fernandez-Herrero L A; Olabarria G; Berenguer J; Blaser M J; **Kuen B**; **Lubitz W**; **Sara M**; Pouwels P H; Kolen C P; Boot H J; **Resch S**
CS Universitat Rostock, Germany.
NC RO1 24145
SO FEMS MICROBIOLOGY REVIEWS, (1997 Jun) 20 (1-2) 47-98. Ref: 197
Journal code: AO4; 8902526. ISSN: 0168-6445.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)

(REVIEW, ACADEMIC)
LA English
FS Priority Journals
OS GENBANK-U38842; GENBANK-X91199; GENBANK-X92752
EM 199710
ED Entered STN: 19971013
Last Updated on STN: 19971013
Entered Medline: 19971001
AB In this chapter we report on the molecular biology of **crystalline** surface layers of different bacterial groups. The limited information indicates that there are many variations on a common theme. Sequence variety, antigenic diversity, gene expression, rearrangements, influence of environmental factors and applied aspects are addressed. There is considerable variety in the S-layer composition, which was elucidated by sequence analysis of the corresponding genes. In Corynebacterium glutamicum one major cell wall protein is responsible for the formation of a highly ordered, hexagonal array. In contrast, two abundant surface proteins from the S-layer of Bacillus anthracis. Each protein possesses three S-layer homology motifs and one protein could be a virulence factor. The antigenic diversity and ABC transporters are important features, which have been studied in methanogenic archaea. The expression of the S-layer components is controlled by three genes in the case of Thermus thermophilus. One has repressor activity on the S-layer gene promoter, the second codes for the **S-layer protein**. The rearrangement by reciprocal recombination was investigated in Campylobacter fetus. 7-8 S-layer proteins with a high degree of homology at the 5' and 3' ends were found. Environmental changes influence the surface properties of Bacillus stearothermophilus. Depending on oxygen supply, this species produces different S-layer proteins. Finally, the molecular bases for some applications are discussed. Recombinant S-layer fusion proteins have been designed for biotechnology.

L19 ANSWER 21 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 14
AN 1997:294708 BIOSIS
DN PREV199799593911
TI Oxygen-triggered synchronised variant formation of the S-layer carrying Bacillus stearothermophilus PV72 during continuous cultivation.
AU Schuster, Kurt C.; Pink, Tomas; Mayer, Harald F.; Hampel, Werner A.; **Sara, Margit (1)**
CS (1) Zentrum Ultrastrukforschung, Ludwig Boltzmann-Inst. Molekulare Nanotechnolgie, Univ. Bodenkultur, Gregor-Mendelstr. 33, 1180 Vienna Austria
SO Journal of Biotechnology, (1997) Vol. 54, No. 1, pp. 15-28.
ISSN: 0168-1656.
DT Article
LA English
AB The **crystalline** cell surface layer (S-layer) of Bacillus stearothermophilus PV72 shows hexagonal lattice symmetry and is composed of a single protein species with a molecular weight of 130 000, termed SbsA. Stable synthesis of the SbsA on synthetic PVIII-medium was achieved during continuous cultivation of the wild-type strain at constant DO of 20%. When the DO was controlled at 50%, an apparent steady state was achieved after 2 volume exchanges after starting continuous culture. After 2-3 further volume exchanges, the respiratoric activity and culture fluorescence peaked markedly while the redox potential showed a steady increase. The increase in the optical density did not correlate with an increase in biomass concentration but rather reflected differences in the morphology of the cells and a slight sporulation. In addition to the physiological and morphological changes, a switch in **S-layer protein** synthesis was observed. Instead of the SbsA from the wild-type strain, an **S-layer protein** with a molecular weight of 97 000, termed SbsB, was produced which assembled into an oblique lattice type. The decrease in the

wild-type **S-layer protein** content in biomass samples harvested during variant formation, freeze-etching and immunogold-labelling of whole cells confirmed that expression of SbsA was synchronously stopped in most, if not in all individual cells of the culture and was completely replaced by synthesis of the SbsB. Peptide mapping, N-terminal sequencing and Western blotting confirmed that both types of S-layer proteins were encoded by different genes. The hypothesis of oxygen-triggered synchronized variant formation was finally supported by computer simulations.

L19 ANSWER 22 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 15
AN 1996:576197 BIOSIS
DN PREV199799290878
TI 2-D Protein crystals as an immobilization matrix for producing reaction zones in dipstick-style immunoassays.
AU Breitwieser, A.; Kupcu, S.; **Howorka, S.**; Weigert, S.; Langer, C.; Hoffmann-Sommergruber, K.; Scheiner, O.; Sleytr, U. B.; **Sara, M.** **(1)**
CS (1) Zentrum fuer Ultrastrukturforschung und Ludwig Boltzmann-Inst. fuer Molekulare Nanotechnologie, Univ. fuer Bodenkultur, Gregor Mendelstrasse 33, A-1180 Vienna Austria
SO Biotechniques, (1996) Vol. 21, No. 5, pp. 918-922, 924-925.
ISSN: 0736-6205.
DT Article
LA English
AB In the present study, the applicability of **crystalline** bacterial cell-surface layers (S-layers) as novel immobilization matrices and reaction zones for dipstick-style immunoassays was investigated. For this purpose, S-layer-carrying cell-wall fragments from Bacillus sphaericus CCM 2120 were deposited on a microporous support, and the **S-layer protein** was cross-linked with glutaraldehyde. For developing appropriate test systems, either human IgG was directly linked to the carboxylic acid groups from the **S-layer protein** or it was immobilized using Protein A or after biotinylation, using streptavidin. A clear correlation was obtained between the amount of anti-human IgG applied and the absorbance values in the immunoassays. S-layers with covalently bound recombinant major birch pollen allergen wee used for quantitative and semiquantitative determination of an antibody raised against it. Using S-layers as an immobilization matrix in comparison to amorphous polymers has advantages in that the closed monolayers of functional macromolecules on their outermost surface allows for strong signals in immunoassays, almost completely eliminates background and prevents diffusion.

L19 ANSWER 23 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 16
AN 1996:262212 BIOSIS
DN PREV199698818341
TI Description of Bacillus thermoaerophilus sp. nov., to include sugar beet isolates and Bacillus brevis ATCC 12990.
AU Meier-Stauffer, Katharina; Busse, Hans-Juergen; Rainey, Frederick A.; Burghardt, Jutta; Scheberl, Andrea; Hollaus, Friedrich; **Kuen, Beatrix**; Makristathis, Athanasios; **Sleytr, Uwe B.**; Messner, Paul (1)
CS (1) Zentrum fuer Ultrastrukturforschung, Universitaet fuer Bodenkultur, Gregor-Mendel-Str. 33, A-1180 Vienna Austria
SO International Journal of Systematic Bacteriology, (1996) Vol. 46, No. 2, pp. 532-541.
ISSN: 0020-7713.
DT Article
LA English
AB Isolates of thermophilic bacteria obtained from an Austrian beet sugar factory were screened by sodium dodecyl sulfate-polyacrylamide gel

electrophoresis (SDS-PAGE) and freeze-fracture electron microscopy for the presence of glycosylated **crystalline** cell surface layers (S-layers). On the basis of similarities in the protein band patterns on SDS-PAGE gels and the lattice geometry of the S-layers as revealed by electron micrographs, the 31 isolates which we studied were clustered into five groups (groups I to V) and several strains which exhibited no common characteristics (group 0). We found that the organisms belonging to groups I to III had glycosylated S-layer proteins, but the highest carbohydrate contents were observed in group III organisms. Partial sequencing of the 16S ribosomal DNAs of selected representative strains of each group revealed that the group I, II, IV, and V isolates and the few group 0 strains were different from the group III strains. The results of DNA-DNA hybridization experiments, SDS-PAGE, and an analysis of polar lipids demonstrated that group III isolates L419-91, L420-91-T (T = type strain), and L438-91 belong to the same species. We chose the group III organism Bacillus sp. strain L420-91-T for further analysis because of the high carbohydrate content of its **S-layer protein**. The taxonomic position of this isolate was determined by using a polyphasic approach. Phenotypic, chemotaxonomic, and genomic analyses revealed that strains L420-91-T, L419-91, and L438-91 represent a new Bacillus species. We observed high levels of similarity between these strains and Bacillus brevis ATCC 12990, which also had a glycosylated **S-layer protein**. Our results show that strains L420-91-T, L419-91, and L438-91 and B. brevis ATCC 12990 belong to the same species and that this species is a new Bacillus species, which we name Bacillus thermoaerophilus. The type strain of this species is strain L420-91 (= DSM 10154).

L19 ANSWER 24 OF 43 CAPLUS COPYRIGHT 2002 ACS
AN 1996:76991 CAPLUS
DN 124:142911
TI Toward selective elicitation of TH1-controlled vaccination responses: vaccine applications of bacterial surface layer proteins
AU Jahn-Schmid, Beatrice; Messner, Paul; Unger, Frank M.; **Sleytr, Uwe B.**; Scheiner, Otto; Kraft, Dietrich
CS Zentrum fuer Ultrastrukturforschung und Ludwig Boltzmann-Institut fuer Molekulare Nanotechnologie, Universitaet fuer Bodenkultur, Vienna, A-1180, Austria
SO J. Biotechnol. (1996), 44(1-3), 225-31
CODEN: JBITD4; ISSN: 0168-1656
DT Journal; General Review
LA English
AB A review with 17 refs. Bacterial surface layer proteins have been utilized as combined vaccine carrier/adjuvants and offer a no. of advantages in these applications. The cryst. protein arrays contain functional groups in precisely defined orientations for coupling of haptens. Conventional applications of S-layer vaccines do not cause observable trauma or side effects. Depending on the nature of the S-layer prepns., antigenic conjugates will induce immune responses of a predominantly cellular or predominantly humoral nature. Immune responses to S-layer-hapten conjugates are also obsd. following oral/nasal application. In the present contribution, the status of investigations with S-layer conjugates in three main immunol. projects is reviewed. In a project aimed at immunotherapy of cancer, conjugates of S-layer with small, tumor-assocd. oligosaccharides have been found to elicit hapten-specific DTH responses. An enlarged program of chem. synthesis has now been initiated to prep. a **complete** set of mucin-derived, tumor-assocd. oligosaccharides and their chem. modified analogs for elicitation of cell-mediated immune responses to certain tumors in humans. In another application, oligosaccharides derived from capsules of Streptococcus pneumoniae type 8 have been linked to S-layer proteins and have been found to elicit protective antibody responses in animals. Most recently, allergen-S-layer conjugates have been prepd. with the intention to suppress the TH2-directed, IgE-mediated allergic responses to Bet.nu.1,

the major allergen of birch pollen. In the former two applications, the S-layer vaccine technol. appears to offer the versatility needed to direct vaccination responses toward predominant control by TH1 or TH2 lymphocytes to meet the different therapeutic or prophylactic requirements in each case. In the third application, work has progressed to a preliminary stage only.

L19 ANSWER 25 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 17
AN 1996:566451 BIOSIS
DN PREV199799295807
TI Regulation of **S-layer protein** synthesis of Bacillus stearothermophilus PV72 through variation of continuous cultivation conditions.
AU Pink, Tomas; Langer, Klemens; Hotzy, Christoph; **Sara, Margit (1)**
CS (1) Zentrum Ultrastrukturforschung, Ludwig Boltzmann-Inst. Mol. Nanotechnol., Univ. Bodenkultur, Gregor-Mendelstr. 33, 1180 Vienna Austria
SO Journal of Biotechnology, (1996) Vol. 50, No. 2-3, pp. 189-200. ISSN: 0168-1656.
DT Article
LA English
AB The **crystalline** cell surface layer (S-layer) of Bacillus stearothermophilus PV72 shows hexagonal lattice symmetry and is composed of a single protein species with a molecular weight of 130000. For investigating the regulation of **S-layer protein** synthesis, Bacillus stearothermophilus PV72 was grown in . continuous culture on synthetic PVIII-medium with glucose as carbon source at constant dilution rate of 0.3 h-1 at 57 degree C under different conditions and limitations. A **complete** outer S-layer and an **S-layer protein** pool sufficient for formation of about 70% inner S-layer was produced under carbon-limited growth. The inner S-layer results from an **S-layer protein** pool stored in the peptidoglycan-containing layer of whole cells which can emerge and assemble on the inner face of the rigid cell wall layer during the cell wall preparation procedure. Under oxygen-limited growth. only a **complete** outer S-layer but no **S-layer protein** pool was synthesized. Reduction of the methionine concentration of PVIII-medium from 100 to 10 mg l-1 led to a clear decrease in **S-layer protein** production and to an incomplete outer S-layer. During growth in the presence of excess glucose, **S-layer protein** synthesis was replaced by that of an exopolysaccharide matrix. After changing to carbon limitation again, the original level of **S-layer protein** synthesis was achieved after only four volume exchanges. Feeding of casein hydrolysate or aromatic or basic amino acids to the continuous culture induced an irreversible loss of **S-layer protein** synthesis after from five to ten volume exchanges. In contrast, addition of Gly, Ala, Val, Leu, Ile, Glu, Gin, Asp, Asn, Ser and Thr in different mixtures could significantly stimulate **S-layer protein** production.

L19 ANSWER 26 OF 43 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 18
AN 96362419 EMBASE
DN 1996362419
TI Ultrafiltration membranes prepared from **crystalline** bacterial cell surface layers as model systems for studying the influence of surface properties on protein adsorption.
AU Weigert S.; **Sara M.**
CS Zentrum fur Ultrastrukturforschung, Universitat fur Bodenkultur, Gregor Mendelstr. 33,1180 Wien, Austria
SO Journal of Membrane Science, (1996) 121/2 (185-196). ISSN: 0376-7388 CODEN: JMESDO
PUI S 0376-7388(96)00176-7
CY Netherlands

DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English
AB **Crystalline** bacterial cell surface layers (S-layers) were used for the preparation of the active filtration layer of ultrafiltration membranes (S-layer ultrafiltration membranes; SUMs). Since the S-layer is uniform in its pore size and morphology and its functional groups are aligned in well-defined positions, the SUMs provide ideal model systems for studying protein adsorption and membrane fouling. Due to the presence of surface-located carboxyl groups the standard SUMs have the net negative charge but exhibit basically a hydrophobic character. In order to change the net charge, the charge density and the accessibility of charged groups of the SUMs as well as their hydrophobicity, free carboxyl groups of the **S-layer protein** were modified with selected low molecular weight nucleophiles under conditions of preserving the **crystalline** lattice structure. SUMs with 1.6 to 7 charged or functional groups exposed per nm2 of the membrane area were used for adsorption experiments. After solutions of differently sized and charged test proteins were filtered, the relative flux losses of distilled particle free water were measured. The results showed that the adsorption capacity of the SUMs increased with the extent of their hydrophobicity. Test proteins showed their own specific adsorption characteristics, which clearly demonstrated the difficulties in determining parameters controlling the membrane fouling. Independent of the net charge of the test proteins and that of the SUMs, the flux loss of SUMs increased with the increased charge density and an improved accessibility of the charged groups on the S-layer surface. No essential differences in the adsorption characteristics were observed between the zwitterionic SUMs of slightly surplus of free carboxyl groups and the standard SUMs of net negative charge.

L19 ANSWER 27 OF 43 CAPLUS COPYRIGHT 2002 ACS
AN 1997:104180 CAPLUS
DN 126:114684
TI **Crystalline** bacterial cell surface layers (S-layers): From cell structure to biomimetics
AU **Sara, M.**; Sleytr, U. B.
CS Center for Ultrastructure Research and Ludwig Boltzmann Institute for Molecular Nonotechnology, Universitat fur Bodenkultur, Vienna, A-1180, Austria
SO Prog. Biophys. Mol. Biol. (1996), 65(1/2), 83-111
CODEN: PBIMAC; ISSN: 0079-6107
PB Elsevier
DT Journal; General Review
LA English
AB A review with 201 refs. on the occurrence, location, ultrastructure, assembly and morphogenesis, mol. biol., functional aspects, and application (biotechnol. application, biomimetics and mol. nanotechnol., and vaccine development) of the title S-layers.

L19 ANSWER 28 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 19
AN 1995:312851 BIOSIS
DN PREV199598327151
TI The **crystalline** cell surface layer from Thermoanaerobacter thermohydrosulfuricus L111-69 as an immobilization matrix: Influence of the morphological properties and the pore size of the matrix on the loss of activity of covalently bound enzymes.
AU Kupcu, Seta; Mader, Christoph; **Sara, Margit (1)**
CS (1) Boitzmann-Inst. Mol. Nanotechnol., Univ. bodenkultur, Gregor-Mendelstrasse 33, 1180 Wien Austria
SO Biotechnology and Applied Biochemistry, (1995) Vol. 21, No. 3, pp. 275-286.

ISSN: 0885-4513.
DT Article
LA English
AB The hexagonally ordered cell surface layer (S-layer) from Thermoanaerobacter thermohydrosulfuricus L111-69 was used as a matrix for the immobilization of naringinase (EC 3.2.1.40, alpha-L-rhamnosidase) (M-r 110000), beta-glucosidase (M-r 66000) and peroxidase (M-r 44000). Naringinase is significantly larger than the 4-5 nm-sized pores passing through the S-layer lattice and could also span the 6 run-wide central funnel-shaped depression in the centre of the hexameric unit cells. The enzyme was immobilized as a monolayer at the outermost surface of the S-layer lattice and retained 60-80% of its original activity. Because of its smaller molecular size, beta-glucosidase was capable of penetrating the central funnel-shaped depression and, therefore, could follow the topography of the S-layer lattice to a greater extent. A tenfold increase in activity from 16 to 160% was observed when beta-glucosidase was immobilized via spacers. Although the 4-5 nm-sized peroxidase could be immobilized via different functional groups in high density, the enzymic activity retained was always less than 3%. Since the **S-layer protein** was also available in monomeric form, soluble **S-layer protein**-peroxidase conjugates with a molar ratio of 3:1 could be prepared in which 40% of activity from the native enzyme was preserved. These comparable studies with soluble and **crystalline S-layer protein** confirmed that rather than the physicochemical properties of the immobilization matrix, the entrapping of the peroxidase molecules inside the pores is responsible for the high activity loss. In summary, the results obtained with a **crystalline** immobilization matrix show that the activity loss of immobilized enzymes correlates with the extent of interactions with the matrix.

L19 ANSWER 29 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 20
AN 1995:320235 BIOSIS
DN PREV199598334535
TI Liposomes coated with **crystalline** bacterial cell surface protein (S-layer) as immobilization structures for macromolecules.
AU Kuepcue, Seta; **Sara, Margit**; **Sleytr, Uwe B. (1)**
CS (1) Zentrum Ultrastrukturforschung Univ. Bodenkultur, Ludwig Boltzmann-Inst. Molekular Nanotechnol., Univ. Bodenkultur, Greogor-Mendelstr. 33, 1180 Wien Austria
SO Biochimica et Biophysica Acta, (1995) Vol. 1235, No. 2, pp. 263-269. ISSN: 0006-3002.
DT Article
LA English
AB Isolated subunits from the **crystalline** cell surface layer (S-layer) of Bacillus coagulans E38-66 were recrystallized on positively charged liposomes. The liposomes were composed of dipalmitoylphosphatidylcholine/cholesterol and stearylamine. The natural arrangement of the S-layer subunits on the bacterial surface is as an oblique (p2) lattice. The subunits attached to positively charged liposomes by their inner face (which bears a net negative charge) in an orientation identical to the lattice on intact cells. The **S-layer protein**, once recrystallized on liposomes, was crosslinked with glutaraldehyde and subsequently used as a matrix for the covalent attachment of macromolecules. The high stability of S-layer-coated liposomes and the possibility for immobilizing biologically active molecules on the **crystalline** array may offer potential in various different liposome applications.

L19 ANSWER 30 OF 43 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 95160447 EMBASE
DN 1995160447
TI Liposomes coated with **crystalline** bacterial cell surface protein

(S- layer) as immobilization structures for macromolecules.
AU Kupcu S.; **Sara M.**; Sleytr U.B.
CS Zentrum fur Ultrastrukturforschung, Universitat fur Bodenkultur, Gregor-Mendelstr. 33,1180 Wien, Austria
SO Biochimica et Biophysica Acta - Biomembranes, (1995) 1235/2 (263-269). ISSN: 0005-2736 CODEN: BBBMBS
CY Netherlands
DT Journal; Article
FS 004 Microbiology
029 Clinical Biochemistry
LA English
SL English
AB Isolated subunits from the **crystalline** cell surface layer (S-layer) of Bacillus coagulans E38-66 were recrystallized on positively charged liposomes. The liposomes were composed of dipalmitoylphosphatidylcholine/cholesterol and stearylamine. The natural arrangement of the S-layer subunits on the bacterial surface is as an oblique (p2) lattice. The subunits attached to positively charged liposomes by their inner face (which bears a net negative charge) in an orientation identical to the lattice on intact cells. The **S-layer protein**, once recrystallized on liposomes, was crosslinked with glutaraldehyde and subsequently used as a matrix for the covalent attachment of macromolecules. The high stability of S-layer-coated liposomes and the possibility for immobilizing biologically active molecules on the **crystalline** array may offer potential in various different liposome applications.

L19 ANSWER 31 OF 43 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 21
AN 95327419 EMBASE
DN 1995327419
TI Surface modification of an ultrafiltration membrane with **crystalline** structure and studies on interactions with selected protein molecules.
AU Weigert S.; **Sara M.**
CS Zentrum fur Ultrastrukturforschung, Universitat fur Bodenkultur, Gregor Mendelstr 33,1180 Wien, Austria
SO Journal of Membrane Science, (1995) 106/1-2 (147-159). ISSN: 0376-7388 CODEN: JMESDO
CY Netherlands
DT Journal; Article
FS 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
LA English
SL English
AB The **crystalline** cell surface layer (S-layer) from the Gram-positive bacterium Bacillus sphaericus CCM 2120 which shows square symmetry and a pore size of 4-5 nm was used for production of ultrafiltration (UF) membranes, termed S-layer ultrafiltration membranes (SUMs). Since S-layers are composed of identical protein or glycoprotein subunits which are arranged according to a 2D protein crystal, pores passing through show identical size and morphology. SUMs were produced by depositing isolated S-layer material on microfiltration membranes, crosslinking the **S-layer protein** with glutaraldehyde and reducing Schiff bases with sodium borohydride. In native S-layer lattices equimolar amounts of amino and carboxyl groups were found to be exposed on surface-located protein domains and in the pore areas. After crosslinking the **S-layer protein** with glutaraldehyde, the S-layer surface and the pores assumed a net negative charge. By using S-layer material in which only the outer face of the S-layer lattice was exposed 1.6 carboxyl groups per nm2 were determined which are relevant for interactions with protein molecules in solutions. For preparing ultrafiltration membranes with **crystalline** structure and high charge density, glutamic acid

residues were stepwise introduced into the S-layer lattice which led to a charge density of either 3.2 or 7.0 carboxyl groups per nm2. Conversion of negatively charged carboxyl groups into free amino groups by preserving the **crystalline** lattice structure was also possible. For obtaining more detailed information on factors inducing flux losses of UF membranes, neutral hydrophilic and neutral hydrophobic, negatively and positively charged SUMs were used for filtration of selected test proteins differing in molecular size and charge. The combination of filtration experiments and high-resolution electron microscopical studies allowed to determine that adsorption of proteins inside the pores with a size similar to the dimension of the test molecules caused considerable flux decline of up to 70%. As derived from the thickness of the S-layer lattice and the molecular dimension of the test proteins, a single layer of protein molecules inside the pores was sufficient for generating measured flux losses. On the contrary, ferritin which is significantly larger than the pores could only adsorb on the outer face of the S-layer lattice but not penetrate the pore openings. Although electron microscopical studies revealed that a monolayer was present on the S-layer surface, flux losses of SUMs were only in the range of 20%.

L19 ANSWER 32 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 22
AN 1995:452659 BIOSIS
DN PREV199598466959
TI A synthetic medium for continuous culture of the S-layer carrying Bacillus stearothermophilus PV 72 and studies on the influence of growth conditions on cell wall properties.
AU Schuster, Kurt Christian; Mayer, Harald F.; Kieweg, Richard; Hampel, Werner A.; **Sara, Margit (1)**
CS (1) Zentrum Ultrastrukturforschung Ludwig Boltzmann-Inst. Molekulare Nanotechnol., Univ. Bodenkultur, Gregor Mendel-Strasse 33, A-1180 Wien Austria
SO Biotechnology and Bioengineering, (1995) Vol. 48, No. 1, pp. 66-77. ISSN: 0006-3592.
DT Article
LA English
AB Bacterial cell surface layers (S-layers) which show a **crystalline** structure, defined pores, and a regular arrangement of functional groups can be used for production of isoporous ultrafiltration membranes and as a matrix for immobilization of macromolecules. S-layer-carrying cell wall fragments from thermophilic Bacillaceae possess an extremely thin peptidoglycan-containing layer with pores larger than those in the S-layer lattice. Thus, they can directly be used for biotechnological applications, when an **S-layer protein** pool is stored in the rigid cell wall layer which is released during cell wall preparation, forming an inner S-layer. In the present study, a synthetic medium for Bacillus stearothermophilus PV 72 was developed by applying the pulse and shift technique with the aim to produce cell wall fragments with beforementioned properties by varying the growth conditions in continuous culture. The organism was grown at 57 degree C in a bioreactor with 1 L working volume equipped with exhaust gas analysis and connected to a PC-based process control system. Biomass concentration was 2.2 g/L out of 8 g/L glucose at a dilution rate of 0.3 h-1, giving a biomass productivity of 0.66 g/L h. Although the organism was grown under different conditions, no change in peptidoglycan composition, extent of peptidoglycan crosslinking, and content of secondary cell wall polymers was observed. The amount of **S-layer protein** pool stored in the rigid cell wall layer and the autolytic activity depended mainly on the specific growth rate. Cell wall fragments with properties required for ultrafiltration membrane production could be produced by parameter settings in continuous culture.

L19 ANSWER 33 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 23

AN 1994:121421 BIOSIS
DN PREV199497134421
TI Novel protein A affinity matrix prepared from two-dimensional protein crystals.
AU Weiner, Christian; **Sara, Margit (1)**; **Sleytr, Uwe B.**
CS (1) Zentrum Ultrastrukturforsch. Ludwig Boltzmann-Inst. Mol. Nanotechnol., Univ. Bodenkultur, Gregor Mendel Str. 33, A-1180 Vienna Austria
SO Biotechnology and Bioengineering, (1994) Vol. 43, No. 4, pp. 321-330. ISSN: 0006-3592.
DT Article
LA English
AB In this article, we describe a novel type of affinity matrix which was prepared by covalently binding Protein A to **crystalline** cell surface layers (S-layers) from the gram-positive Clostridium thermohydrosulfuricum L111-69. S-layers were used in the form of cell wall fragments, which were obtained by breaking whole cells by ultrasonification and removing the cell content and the plasma membrane. In these thimble-shaped structures, revealing a size of 1 to 2 mu-m, the peptidoglycan-containing layer was covered on both faces with a hexagonally ordered S-layer lattice composed of identical glycoprotein subunits. After crosslinking the **S-layer protein** with glutaraldehyde, carboxyl groups from acidic amino acids were activated with carbodiimide and used for immobilization of Protein A. Quantitative determination confirmed that up to two Protein A molecules were bound per S-layer subunit leading to a dense monomolecular coverage of the immobilization matrix with the ligand. Affinity microparticles were capable of adsorbing IgG from solutions of purified preparations, from artificial IgG-albumin mixtures, and from serum. The amount of IgG bound to affinity microparticles corresponded to the theoretical saturation capacity. Under appropriate conditions, up to 95% of the adsorbed IgG could be eluted again. Affinity microparticles were found to have an extremely low Protein A leakage and a high stability toward mechanical forces. Because pores in the S-layer lattice revealed a size of 4 to 5 nm, immobilization of Protein A and adsorption of IgG was restricted to the outermost surface area. This excludes mass transfer problems usually encountered with affinity matrices prepared from amorphous polymers where more than 90% of the ligands are immobilized in the interior.

L19 ANSWER 34 OF 43 CAPLUS COPYRIGHT 2002 ACS
AN 1993:623656 CAPLUS
DN 119:223656
TI Induction of T-cell immunity to oligosaccharide antigens immobilized on **crystalline** bacterial surface layers (S-layers)
AU Smith, Richard H.; Messner, Paul; Lamontagne, Louis R.; **Sleytr, Uwe B.**; Unger, Frank M.
CS Chembiomed Ltd., Edmonton, AB, T6H 4N9, Can.
SO Vaccine (1993), 11(9), 919-24
CODEN: VACCDE; ISSN: 0264-410X
DT Journal
LA English
AB Immunization of Balb/c mice with conjugates of oligosaccharide haptens and cryst. bacterial surface-layer proteins (S-layers) primed the mice for a strong, hapten-specific, delayed-type hypersensitivity (DTH) response. Conjugates of haptens with bovine serum albumin produced only weak DTH responses but, when mixed with aluminum hydroxide, elicited DTH responses comparable to those against S-layer conjugates. Surface-layer conjugates also elicited strong anti-hapten DTH responses when administered by an oral/ nasal route. Apparently, the natural assembly of S-layer proteins into large, two-dimensional arrays endows them with intrinsic adjuvant properties.

L19 ANSWER 35 OF 43 CAPLUS COPYRIGHT 2002 ACS
AN 1994:6257 CAPLUS

DN 120:6257
TI **Crystalline** bacterial cell surface layers (S-layers) as combined carrier/adjuvants for conjugate vaccines
AU Malcolm, A. J.; Messner, P.; Sleytr, U. B.; Smith, R. H.; Unger, F. M.
CS Alberta Res. Counc., Edmonton, AB, T6H 5X2, Can.
SO Immobilised Macromol. (1993), 195-207. Editor(s): **Sleytr, Uwe Bernd**. Publisher: Springer, London, UK.
CODEN: 59IKAS
DT Conference; General Review
LA English
AB A review with .apprx.40 refs. S-layers are 2-dimensional cryst. arrays of protein subunits forming surface layers on prokaryotic cells. Most archaebacteria and some eubacteria form their layers. The prepn. and testing of conjugate vaccines using S-layers as carriers for weakly immunogenic carbohydrate antigen and haptens are described.

L19 ANSWER 36 OF 43 CAPLUS COPYRIGHT 2002 ACS
AN 1993:187221 CAPLUS
DN 118:187221
TI Influence of covalent attachment of low-molecular-weight substances on the rejection and adsorption properties of **crystalline** proteinaceous ultrafiltration membranes
AU Kupcu, Seta; **Sara, Margit; Sleytr, Uwe B.**
CS Inst. Mol. Nanotechnol., Univ. Bodenkultur, Vienna, 1180, Austria
SO Desalination (1993), 90(1-3), 65-76
CODEN: DSLNAH; ISSN: 0011-9164
DT Journal
LA English
AB Many bacteria possess cryst. cell surface layers (S-layers) as their outermost cell envelope component. High-resoln. electron microscopical and permeability studies on S-layers revealed pores with a size of 2-8 nm. Each type of S-layer lattice showed pores of identical size and morphol. By depositing S-layer fragments on appropriate microfiltration membranes and crosslinking the **S-layer protein** with glutaraldehyde, it was possible to produce ultrafiltration membranes with a cryst. and isoporous active filtration layer. Due to a surplus of free carboxyl groups on the surface of the S-layer lattice and in the pore areas, these composite S-layer ultrafiltration membranes (SUMs) showed a net neg. charge. Upon converting the free carboxyl groups into neutral or pos. charged groups by amidation, both the adsorption and rejection properties of SUMs could be altered. As expected, neutral SUMs showed the lowest unspecific protein adsorption. Membrane modification studies further demonstrated that in addn. to the mol. size, the physicochem. properties of the immobilized compds. and the resulting interactions with the test proteins in soln. det. the rejection characteristics of SUMs.

L19 ANSWER 37 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 24
AN 1993:94036 BIOSIS
DN PREV199395049232
TI Introduction of sulphydryl groups into the **crystalline** bacterial cell surface layer protein from Bacillus stearothermophilus PV72 and its application as an immobilization matrix.
AU **Sara, Margit (1); Sleytr, Uwe B.**
CS (1) Zentrum Ultrastrukturforschung, Ludwig Boltzmann-Inst. Molekulare Nanotechnologie, Univ. Bodenkultur, Gregor-Mendel-Str. 33, A-1180 Vienna Austria
SO Applied Microbiology and Biotechnology, (1992) Vol. 38, No. 2, pp. 147-151.
ISSN: 0175-7598.
DT Article
LA English
AB The **crystalline** cell surface layer (S-layer) from Bacillus stearothermophilus PV72 was used as a matrix for reversible immobilization

of beta-D-galacosidase via disulphide bonds. In order to obtain an immobilization matrix stable towards acid, alkali and reducing agents such as dithiothreitol (DTT), the S-layer subunits were first cross-linked with glutaraldehyde. This was done in a way whereby 75% of the free amino groups remained unmodified, and then could be completely converted into sulphhydryl groups upon reaction with the monofunctional imidoester iminothiolane. After activation of the sulphhydryl groups with 2,2'-dipyridyldisulphide, 550 mu-g beta-D-galactosidase could be immobilized per milligram of **S-layer protein**, which corresponds to one beta-D-galactosidase molecular (relative molecular mass (M-r), 1160 00) per two S-layer subunits (M-r, 130000). At least 90% of the sulphhydryl groups from the **S-layer protein** could be regenerated for further activation by cleaving the disulphide bonds with DTT. In comparative studies beta-D-galactosidase was linked to carbodiimide-activated carboxyl groups of the **S-layer protein**.

L19 ANSWER 38 OF 43 USPATFULL
AN 91:68711 USPATFULL
TI Immunogenic compositions containing ordered carriers
IN **Sleytr, Uwe B.**, Vienna, Austria
Mundt, Wolfgang, Vienna, Austria
Messner, Paul, Vienna, Austria
Smith, Richard H., Edmonton, Canada
Unger, Frank M., Edmonton, Canada
PA Chembiomed, Ltd., Edmonton, Canada (non-U.S. corporation)
PI US 5043158 19910827
AI US 1990-487729 19900302 (7)
RLI Continuation-in-part of Ser. No. US 1988-233674, filed on 18 Aug 1988, now abandoned
PRAI DE 1987-3717897 19870821
CA 1988-575340 19880822
DT Utility
FS Granted
EXNAM Primary Examiner: Russel, Jeffrey E.; Assistant Examiner: Kim, Kay
LREP Irell & Manella
CLMN Number of Claims: 6
ECL Exemplary Claim: 1
DRWN 7 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1000
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Superior pharmaceutical compositions which comprise carriers coupled to epitope-bearing moieties are described. The carriers are **crystalline** or paracrystalline glycoproteins, especially those derived from S-layers of microbial cell walls. These conjugates are capable of eliciting the formation of antibodies as well as a T-cell response.

L19 ANSWER 39 OF 43 USPATFULL
AN 91:52276 USPATFULL
TI Use of structure with membrane having continuous pores
IN **Sleytr, Uwe**, 10 Parhamerplatz, A-1170 Vienna, Austria
Sara, Margit, 90/2/24 Vorgartenstr, A-1200 Vienna, Austria
PI US 5028335 19910702
AI US 1989-348779 19890508 (7)
RLI Continuation-in-part of Ser. No. US 1988-174127, filed on 28 Mar 1988, now patented, Pat. No. US 4849109, issued on 28 Apr 1989 which is a continuation-in-part of Ser. No. US 1985-795349, filed on 28 Oct 1985, now patented, Pat. No. US 4752395, issued on 28 Jun 1988
PRAI AT 1984-797 19840309
DT Utility
FS Granted
EXNAM Primary Examiner: Spear, Frank
LREP Bierman and Muserlian

CLMN Number of Claims: 30
ECL Exemplary Claim: 3,20
DRWN 16 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 1039
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Use of novel membrane crystal lattice structures with continuous pores as ultrafiltration membranes, gas separating organs and separating organs for ion exchange processes, structure for immobilizing molecules, and films as enveloping material for substances.

L19 ANSWER 40 OF 43 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN 1991-01790 BIOTECHDS
TI **Crystalline** bacterial cell surface layers used as ultrafiltration membranes and immobilization matrix; bacterium S-layer isolation; potential application e.g. as an enzyme support
AU **Sara M**; Kupcu S; Sleytr U B
LO Zentrum fuer Ultrastrukturforschung Universitaet fuer Bodenkultur Gregor-Mendelstr. 33, 1180 Wien, Austria.
SO Genet.Eng.Biotechnol.; (1990) 10, 1, 10-13
DT Journal
LA English
AB Many bacteria have 'periodic macromolecular monolayers' of (glyco) proteins on the outer surface (S-layers). These may be useful in biotechnological applications, such as ultrafiltration membranes or as a support for immobilization of proteins. In S-layer ultrafiltration membranes (SUM), the selective filtration layer consists of coherent S-layer fragments bound to a microfiltration membrane. To enhance mechanical and chemical stability, the **S-layer protein** is crosslinked with glutaraldehyde. SUMs differ from synthetic ultrafiltration membranes in having a **crystalline** and isoporous active filtration layer. SUMs have been prepared from e.g. Bacillus stearothermophilus, and show differences between mesophilic and thermophilic strains. SUMs are stable under cross-flow filtration conditions. Immobilization of e.g. beta-D-fructofuranosidase (EC-3.2.1.26) or glucose-oxidase (EC-1.1.3.4) on S-layers may be carried out by binding to carboxyl groups, introduced disulfide bonds or to exposed carbohydrate chains. Immobilized oxidases on SUMs forming a closed monolayer may be used in biosensor development. (31 ref)

L19 ANSWER 41 OF 43 USPATFULL
AN 89:58419 USPATFULL
TI Use of structure with membrane having continuous pores
IN **Sleytr, Uwe**, 10 Parhamerplatz, A-1170 Vienna, Austria
  Sara, Margit, 90/2/24 Vorgartenstr, A-1200 Vienna, Austria
PI US 4849109 19890718
AI US 1988-174127 19880328 (7)
RLI Continuation-in-part of Ser. No. US 1985-795349, filed on 28 Oct 1985, now patented, Pat. No. US 4752395
PRAI AT 1984-797 19840309
DT Utility
FS Granted
EXNAM Primary Examiner: Jones, W. Gary
LREP Bierman and Muserlian
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 16 Drawing Figure(s); 6 Drawing Page(s)
LN.CNT 954
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Use of novel membrane crystal lattice structures with continuous pores as ultrafiltration membranes, gas separating organs and separating organs for ion exchange processes structure for immobilizing molecules, and films as enveloping material for substances.

L19 ANSWER 42 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 25
AN 1987:338678 BIOSIS
DN BA84:47621
TI CHARGE DISTRIBUTION ON THE S LAYER OF BACILLUS-STEAROTHERMOPHILUS NRS 1536-3C AND IMPORTANCE OF CHARGED GROUPS FOR MORPHOGENESIS AND FUNCTION.
AU **SARA M**; SLEYTR U B
CS ZENTRUM ULTRASTRUKURTFORSCHUNG AND LUDWIG BOLTZMANN INST. ULTRASTRUKTURFORSCHUNG, UNIV. BODENKULTUR, A-1180 VIENNA, AUSTRIA.
SO J BACTERIOL, (1987) 169 (6), 2804-2809.
CODEN: JOBAAY. ISSN: 0021-9193.
FS BA; OLD
LA English
AB The distribution and functional significance of charged groups on the outer and inner faces of the S layer from Bacillus stearothermophilus NRS 1563/3c was investigated. Chemical modification of the exposed amino or carboxyl groups was performed on whole cells, isolated S layers self-assembled in vitro, and cell wall fragments (S layer attached to the peptidoglycan-containing sacculus). Without chemical modification, S layer self-assembly products could be labeled with polycationic ferritin, while S layers on whole cells could not. Following treatment with glutaraldehyde, whole cells were uniformly labeled with polycationic ferritin. Whole cells treated with glutaraldehyde and glycine methyl ester in the presence of carbodiimide did not bind polycationic ferritin significantly above background. Treatment of cell wall fragments with amino-specific, homobifunctional cross-linkers or with carbodiimide alone rendered the **S layer protein** nonextractable with sodium dodecyl sulfate. After amidation of the accessible carboxyl groups, the modified, guanidine hydrochloride-extractable S layer protomers did not self-assemble into regularly structured lattices. N-Amidination with ethylacetimidate did not interfere with the self-assembly of the isolated protomers. N-Acetylation resulted in a considerable destabilization of the S layer lattice, as seen by the release of a large amount of modified protomers during the reaction. N-Succinylation led to a **complete** disintegration of the protein lattice. These results indicated that only the inner face of the S layer carried a net negative charge. On both faces, free amino and carboxyl groups of adjacent protomers were arranged in proximity so as to contribute by electrostatic interactions to the cohesion of the protomers in the two-dimensional array. The native charge of the protomers was required for both the in vitro self-assembly of the isolated subunits and the maintenance of the structural integrity of the S layer lattice. Among other functions, the biological significance of S layers may be in masking the electronegative charge of the cell wall proper.

L19 ANSWER 43 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 26
AN 1985:410475 BIOSIS
DN BA80:80467
TI STABILITY AND SELF-ASSEMBLY OF THE **S-LAYER PROTEIN** OF THE CELL WALL OF BACILLUS-STEAROTHERMOPHILUS.
AU JAENICKE R; WELSCH R; **SARA M**; SLEYTR U B
CS INST. BIOPHYSIK PHYS. BIOCHEM., UNIVERSITAETSSTRASSE 31, D-8400 REGENSBURG.
SO BIOL CHEM HOPPE-SEYLER, (1985) 366 (7), 663-670.
CODEN: BCHSEI.
FS BA; OLD
LA English
AB The surface layer of the cell envelope of B. stearothermophilus consists of a regular array of protein subunits. As shown by sodium dodecyl sulfate polyacrylamide gel-electrophoresis and ultracentrifugation, the fully solubilized **S-layer protein** represents a homogeneous entity with a subunit MW of 115 .+-. 5 kilodalton. Solubilization of the protein may be accomplished at acid pH, or using

high concentrations of urea or guanidine .cntdot. HCl. It is accompanied by (partial) denaturation, thus interfering with the characterization of the protein in its unperturbed native state. Removal of the solubulizing agent by dialysis or dilution allows the the S-layer to be reassembled into 2-dimensional **crystalline** lattices identical to those observed in intact cells. To determine the kinetics of association, optimum conditions are found to be rapid mixing with 0.1 M sodium phosphate pH 7.0, 20.degree. C, final protein concentration > 10 .mu.g/ml. If the time course of the self-assembly is monitored by light scattering, and by chemical cross-linking with glutardialdehyde, multiphasic kinetics with a rapid initial phase and slow consecutive processes of higher than 2nd-order are observed. The rapid phase may be attributed to the formation of oligomeric precursors (MW > 106). Concentration-dependent light scattering measurements give evidence for a critical concentration of association, suggesting that patches of 12-16 protein subunits fuse and recrystallize into the final (native) S-layer structure. Recrystallization tends to be **complete**.

=> d clm 3

L19 ANSWER 3 OF 43 USPATFULL

CLM What is claimed is:

1. A method of producing a structured layer of defined functional molecules on the upper face of a sheet substrate, characterised in that on the upper face of the substrate structures are produced with different surface characteristics at least in relation to their hydrophobicity, in that a monolayer of protein-containing **crystalline** cell surface layer (S-layer) is deposited by recrystallisation on this structured surface, wherein the S-layer is only deposited at the structured regions of the surface with increased hydrophobicity, and in that the functional molecules are deposited or intercalated at the surface or into the interspaces of the crystal lattice of the S-layer.

2. A method according to claim 1, characterised in that the surface of a substrate is divided into hydrophobic areas on the one hand and hydrophilic areas on the other, by coating this substrate and structuring this coating by removing the coating in predefined regions, wherein the subsequent deposition of the S-layer by recrystallisation is performed on the hydrophobic areas.

3. A method according to claim 1, characterised in that the surface of a substrate is structured into regions with hydrophobic properties and regions with hydrophilic properties by exposure to radiation with predefined intensity and energy, for example masked ion radiation, wherein the subsequent deposition of the S-layer by recrystallisation is only performed in the hydrophobic areas of the substrate surface.

4. A method according to claim 2, characterised in that the substrate is a silicon wafer with an oxide layer structured by removing predefined regions, and the silicon surface of these regions is transferred into an hydrophilic state, whereas those regions with the oxide layer remain in the hydrophobic state and provide for deposition of the S-layer.

5. The method of claim 4 characterized in that the transfer of the silicon surface into an hydrophilic state is accomplished using the RCA-cleaning method.

=> d his

(FILE 'HOME' ENTERED AT 10:28:46 ON 10 MAY 2002)

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      FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS,
      LIFESCI, CAPLUS, USPATFULL, USPAT2' ENTERED AT 10:29:15 ON 10 MAY 2002
                E LUBITZ WERNER/AU
L1          842 S E1-E3
                E SLEYTR UWE/AU
L2          235 S E1-E5
                E SLEYTR U/AU
L3           44 S E3
                E KUEN BEATRIX/AU
L4           27 S E1-E3
                E KUEN B/AU
L5           48 S E3
                E TRUPPE MICHAELA/AU
L6           13 S E1-E4
                E HOWORKA STEFAN/AU
L7           20 S E3-E5
                E HOWORKA S/AU
L8           53 S E3-E8
                E RESCH STEPHANKA/AU
L9            5 S E1-E2
L10          86 S RESCH S/AU
                E RESCH S/AU
L11          86 S E3
                E SCHROLL GERHARD/AU
L12          12 S E3
                E SCHROLL G/AU
L13          43 S E3
                E SARA MARGIT/AU
L14         150 S E1-E3
                E SARA M/AU
L15         378 S E3-E4
L16        1634 S L1-L15
L17         258 S L16 AND S LAYER PROTEIN
L18         118 S  L17 AND (MATURE OR COMPLETE OR CRYSTALLINE)
L19          43 DUP REM L18 (75 DUPLICATES REMOVED)

=> s l18 and (fusion or heterologous or recombinant)
L20          21 L18 AND (FUSION OR HETEROLOGOUS OR RECOMBINANT)

=> dup rem l20
PROCESSING COMPLETED FOR L20
L21           7 DUP REM L20 (14 DUPLICATES REMOVED)

=> d bib ab 1-7

L21  ANSWER 1 OF 7     MEDLINE                              DUPLICATE 1
AN   2002200210     IN-PROCESS
DN   21930707   PubMed ID: 11932495
TI   A recombinant bacterial cell surface (S-layer)-major birch
     pollen allergen-fusion protein (rSbsC/Bet v1) maintains the
     ability to self-assemble into regularly structured monomolecular lattices
     and the functionality of the allergen.
AU   Breitwieser Andreas; Egelseer Eva M; Moll Dieter; Ilk Nicola; Hotzy
     Christoph; Bohle Barbara; Ebner Christof; Sleytr Uwe B;
     Sara Margit
CS   Center for Ultrastructure Research and Ludwig Boltzmann-Institute for
     Molecular Nanotechnology, University of Agricultural Sciences, Gregor
     Mendelstrasse 33, 1180 Vienna and Institute of Pathophysiology, University
     of Vienna, 1090 Vienna, Austria.
SO   PROTEIN ENGINEERING, (2002 Mar) 15 (3) 243-9.
     Journal code: 8801484. ISSN: 0269-2139.
CY   England: United Kingdom
DT   Journal; Article; (JOURNAL ARTICLE)
```

LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020405
Last Updated on STN: 20020405
AB The **mature crystalline** bacterial cell surface (**S-layer**) **protein** SbsC of Bacillus stearothermophilus ATCC 12980 comprises amino acids 31-1099 and assembles into an oblique lattice type. As the deletion of up to 179 C-terminal amino acids did not interfere with the self-assembly properties of SbsC, the sequence encoding the major birch pollen allergen (Bet v1) was fused to the sequence encoding the truncated form rSbsC(31-920). The S-layer **fusion** protein, termed rSbsC/Bet v1, maintained the ability to self-assemble into flat sheets and open-ended cylinders. The presence and the functionality of the fused Bet v1 sequence was proved by blot experiments using BIP1, a monoclonal antibody against Bet v1 and Bet v1-specific IgE-containing serum samples from birch pollen allergic patients. The location and accessibility of the allergen moiety on the outer surface of the S-layer lattice were demonstrated by immunogold labeling of the rSbsC/Bet v1 monolayer, which was obtained by oriented recrystallization of the S-layer **fusion** protein on native cell wall sacculi. Thereby, the specific interactions between the N-terminal part of SbsC and a distinct type of secondary cell wall polymer were exploited. This is the first S-layer **fusion** protein described that had retained the specific properties of the **S-layer protein** moiety in addition to those of the fused functional peptide sequence.

L21 ANSWER 2 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2001:289673 BIOSIS
DN PREV200100289673
TI Analysis of the structure-function relationship of the **S-layer protein** SbsC of Bacillus stearothermophilus ATCC 12980 by producing truncated forms.
AU Jarosch, Marina; Egelseer, Eva M.; Huber, Carina; Moll, Dieter; Mattanovich, Diethard; **Sleytr, Uwe B.**; **Sara, Margit** (1)
CS (1) Centre for Ultrastructure Research, Ludwig Boltzmann-Institute for Molecular Nanotechnology, University of Agricultural Sciences, 1180, Vienna: sara@edv1.boku.ac.at Austria
SO Microbiology (Reading), (May, 2001) Vol. 147, No. 5, pp. 1353-1363. print. ISSN: 1350-0872.
DT Article
LA English
SL English
AB The **mature** surface layer (**S-layer**) **protein** SbsC of Bacillus stearothermophilus ATCC 12980 comprises amino acids 31-1099 and self-assembles into an oblique lattice type which functions as an adhesion site for a cell-associated high-molecular-mass exoamylase. To elucidate the structure-function relationship of distinct segments of SbsC, three N- and seven C-terminal truncations were produced in a **heterologous** expression system, isolated, purified and their properties compared with those of the **recombinant mature S-layer protein** rSbsC31-1099.
With the various truncated forms it could be demonstrated that the N-terminal part (aa 31-257) is responsible for anchoring the S-layer subunits via a distinct type of secondary cell wall polymer to the rigid cell wall layer, but this positively charged segment is not required for the self-assembly of SbsC, nor for generating the oblique lattice structure. If present, the N-terminal part leads to the formation of in vitro double-layer self-assembly products. Affinity studies further showed that the N-terminal part includes an exoamylase-binding site. Interestingly, the N-terminal part carries two sequences of 6 and 7 aa (AKAALD and KAAYEAA) that were also identified on the amylase-binding protein AbpA of Streptococcus gordonii. In contrast to the self-assembling N-terminal truncation rSbsC258-1099, two further N-terminal truncations

(rSbsC343-1099, rSbsC447-1099) and three C-terminal truncations (rSbsC31-713, rSbsC31-844, rSbsC31-860) had lost the ability to self-assemble and stayed in the water-soluble state. Studies with the self-assembling C-terminal truncations rSbsC31-880, rSbsC31-900 and rSbsC31-920 revealed that the C-terminal 219 aa can be deleted without interfering with the self-assembly process, while the C-terminal 179 aa are not required for the formation of the oblique lattice structure.

L21 ANSWER 3 OF 7 MEDLINE DUPLICATE 3
AN 1999204063 MEDLINE
DN 99204063 PubMed ID: 10188248
TI Self-assembly product formation of the Bacillus stearothermophilus PV72/p6 **S-layer protein** SbsA in the course of autolysis of Bacillus subtilis.
AU **Howorka S; Sara M; Lubitz W; Kuen B**
CS Institut fur Mikrobiologie and Genetik, Universitat Wien, Vienna, Austria.
SO FEMS MICROBIOLOGY LETTERS, (1999 Mar 15) 172 (2) 187-96.
Journal code: FML; 7705721. ISSN: 0378-1097.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199904
ED Entered STN: 19990511
Last Updated on STN: 19990511
Entered Medline: 19990429
AB In order to achieve high level expression and to study the release of a protein capable of self-assembly, the gene encoding the **crystalline** cell surface (**S-layer**) **protein** SbsA of Bacillus stearothermophilus PV72/p6, including its signal sequence, was cloned and expressed in Bacillus subtilis. To obtain high level expression, a tightly regulated, xylose-inducible, stably replicating multicopy-plasmid vector was constructed. After induction of expression, the **S-layer protein** made up about 15% of the total cellular protein content, which was comparable to the SbsA content of B. stearothermophilus PV72/p6 cells. During all growth stages, SbsA was poorly secreted to the ambient cellular environment by B. subtilis. Extraction of whole cells with guanidine hydrochloride showed that in late stationary growth phase cells 65% of the synthesised SbsA was retained in the peptidoglycan-containing layer, indicating that the rigid cell wall layer was a barrier for efficient SbsA secretion. Electron microscopic investigation revealed that SbsA release from the peptidoglycan-containing layer started in the late stationary growth phase at distinct sites at the cell surface leading to the formation of extracellular self-assembly products which did not adhere to the cell wall surface. In addition, intracellular sheet-like SbsA self-assembly products which followed the curvature of the cell became visible in partly lysed cells. Intracellularly formed self-assembly products remained intact even after **complete** lysis of the rigid cell envelope layer.

L21 ANSWER 4 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 1998:434363 BIOSIS
DN PREV199800434363
TI A novel dipstick developed for rapid Bet v 1-specific IgE detection: **Recombinant** allergen immobilized via a monoclonal antibody to **crystalline** bacterial cell-surface layers.
AU Breitwieser, A. (1); Mader, C. (1); Schocher, I. (1); Hoffmann-Sommergruber, K.; Aberer, W.; Scheiner, O.; Sleytr, U. B.; **Sara, M. (1)**
CS (1) Zentrum fuer Ultrastrukturforschung, Univ Bodenkultur, Gregor-Mendelstr. 33, 1180 Wien Austria
SO Allergy (Copenhagen), (Aug., 1998) Vol. 53, No. 8, pp. 786-793.
ISSN: 0105-4538.
DT Article

LA English
AB The incidence of allergy to airborne proteins derived from tree and grass pollen, feces of mites, spores of molds, and pet dander has been increasing over the last decades. Since precise diagnosis is a prerequisite for successful immunotherapy, there is a rising demand for rapid, reliable, and inexpensive screening methods such as dipstick assays. With the purified **recombinant** major birch-pollen allergen rBet v 1a as model protein, **crystalline** bacterial cell-surface layers (S-layers) were tested for their applicability as an immobilization matrix for dipstick development. For this purpose, S-layers were deposited on a mechanically stable microporous support, cross-linked with glutaraldehyde, and free carboxylic acid groups of the **S-layer protein** were activated with carbodiimide. In the present test system, rBet v 1a was immobilized via the monoclonal mouse antibody BIP 1, which, unlike the allergen, is too large to enter the pores of the S-layer lattice, and which therefore formed a closed monolayer on the outermost surface of the crystal lattice. Moreover, BIP 1 is known to modulate IgE binding to the allergen. After incubation of the dipsticks in serum, washing of the reaction zone under tap water, and binding of an anti-IgE alkaline phosphatase conjugate, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium was used as substrate, forming an IgE concentration-dependent colored precipitate on the S-layer surface. The investigation of patient sera previously tested with the CAP system confirmed the specificity of the S-layer-based dipstick assay. Since the dipstick is easy to handle and the whole test procedure takes only 90 min, this test system should be applicable for rapid determination of specific IgE and for first screening in the doctor's practice.

L21 ANSWER 5 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:87452 BIOSIS
DN PREV199800087452
TI Bet v 1, the major birch pollen allergen, conjugated to **crystalline** bacterial cell surface proteins, expands allergen-specific T cells of the Th1/Th0 phenotype in vitro by induction of IL-12.
AU Jahn-Schmid, Beatrice; Siemann, Ute; Zenker, Andrea; Bohle, Barbara; Messner, Paul; Unger, Frank M.; **Sleytr, Uwe B.**; Scheiner, Otto; Kraft, Dietrich; Ebner, Christof (1)
CS (1) Inst. Allgemeine Experimentelle Pathologie, Univ. Wien, AKH-EWB-OST 3Q, Waehringer Guertel 18-20, 1090 Wien Austria
SO International Immunology, (Dec., 1997) Vol. 9, No. 12, pp. 1867-1874. ISSN: 0953-8178.
DT Article
LA English
AB Modulation of allergic immune responses by using adequate adjuvants is a promising concept for future immunotherapy of type I hypersensitivity. In the present study, **recombinant** Bet v 1 (rBet v 1, the major birch pollen allergen) was conjugated to cross-linked **crystalline** surface layer proteins (SL) derived from Gram-positive eubacteria. T cell lines (TCL) and clones (TCC) were established from peripheral blood of birch pollen-allergic patients. TCL and TCC were induced either using rBet v 1 alone or rBet v 1/SL conjugates (rBet v 1/SL) as initial antigen stimulus. Cytokine production after re-stimulation with rBet v 1 was investigated. TCL initiated with rBet v 1/SL showed significantly increased IFN-gamma production as compared to rBet v 1-selected TCL. TCC were established from TCL of five patients. As expected, the majority of CD4+ TCC induced by rBet v 1 (55%) displayed a Th2-like pattern of cytokine production. However, only 21% of Bet v 1 -specific TCC isolated from TCL established with the Bet v 1/SL revealed this phenotype. The majority of SL-specific TCC (80%) belonged to the Th1 phenotype. In cultures of peripheral blood mononuclear cells, both, SL and Bet v 1/SL (but not rBet v 1) stimulated the production of high levels of IL-12, a pivotal mediator of Th1 responses. Moreover, stimulation of rBet v

1-induced TCC with rBet v 1/SL led to an Increased IFN-gamma production. This effect could be reversed by neutralizing anti-IL-12 mAb. Together these results indicate an adjuvant effect of SL mediated by IL-12. Our results indicate that bacterial components, such as SL, displaying adjuvant effects may be suitable for immunotherapeutical vaccines for type I allergy.

L21 ANSWER 6 OF 7 MEDLINE DUPLICATE 5
AN 97422863 MEDLINE
DN 97422863 PubMed ID: 9276928
TI Molecular biology of S-layers.
AU Bahl H; Scholz H; Bayan N; Chami M; Leblon G; Gulik-Krzywicki T; Shechter E; Fouet A; Mesnage S; Tosi-Couture E; Gounon P; Mock M; Conway de Macario E; Macario A J; Fernandez-Herrero L A; Olabarria G; Berenguer J; Blaser M J; **Kuen B**; **Lubitz W**; **Sara M**; Pouwels P H; Kolen C P; Boot H J; **Resch S**
CS Universitat Rostock, Germany.
NC RO1 24145
SO FEMS MICROBIOLOGY REVIEWS, (1997 Jun) 20 (1-2) 47-98. Ref: 197
Journal code: AO4; 8902526. ISSN: 0168-6445.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LA English
FS Priority Journals
OS GENBANK-U38842; GENBANK-X91199; GENBANK-X92752
EM 199710
ED Entered STN: 19971013
Last Updated on STN: 19971013
Entered Medline: 19971001
AB In this chapter we report on the molecular biology of **crystalline** surface layers of different bacterial groups. The limited information indicates that there are many variations on a common theme. Sequence variety, antigenic diversity, gene expression, rearrangements, influence of environmental factors and applied aspects are addressed. There is considerable variety in the S-layer composition, which was elucidated by sequence analysis of the corresponding genes. In Corynebacterium glutamicum one major cell wall protein is responsible for the formation of a highly ordered, hexagonal array. In contrast, two abundant surface proteins from the S-layer of Bacillus anthracis. Each protein possesses three S-layer homology motifs and one protein could be a virulence factor. The antigenic diversity and ABC transporters are important features, which have been studied in methanogenic archaea. The expression of the S-layer components is controlled by three genes in the case of Thermus thermophilus. One has repressor activity on the S-layer gene promoter, the second codes for the **S-layer protein**. The rearrangement by reciprocal recombination was investigated in Campylobacter fetus. 7-8 S-layer proteins with a high degree of homology at the 5' and 3' ends were found. Environmental changes influence the surface properties of Bacillus stearothermophilus. Depending on oxygen supply, this species produces different S-layer proteins. Finally, the molecular bases for some applications are discussed. **Recombinant** S-layer **fusion** proteins have been designed for biotechnology.

L21 ANSWER 7 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 1996:576197 BIOSIS
DN PREV199799290878
TI 2-D Protein crystals as an immobilization matrix for producing reaction zones in dipstick-style immunoassays.
AU Breitwieser, A.; Kupcu, S.; **Howorka, S.**; Weigert, S.; Langer, C.; Hoffmann-Sommergruber, K.; Scheiner, O.; Sleytr, U. B.; **Sara, M.** **(1)**
CS (1) Zentrum fuer Ultrastrukturforschung und Ludwig Boltzmann-Inst. fuer

Molekulare Nanotechnologie, Univ. fuer Bodenkultur, Gregor Mendelstrasse 33, A-1180 Vienna Austria
SO Biotechniques, (1996) Vol. 21, No. 5, pp. 918-922, 924-925.
ISSN: 0736-6205.
DT Article
LA English
AB In the present study, the applicability of **crystalline** bacterial cell-surface layers (S-layers) as novel immobilization matrices and reaction zones for dipstick-style immunoassays was investigated. For this purpose, S-layer-carrying cell-wall fragments from Bacillus sphaericus CCM 2120 were deposited on a microporous support, and the **S-layer protein** was cross-linked with glutaraldehyde. For developing appropriate test systems, either human IgG was directly linked to the carboxylic acid groups from the **S-layer protein** or it was immobilized using Protein A or after biotinylation, using streptavidin. A clear correlation was obtained between the amount of anti-human IgG applied and the absorbance values in the immunoassays. S-layers with covalently bound **recombinant** major birch pollen allergen wee used for quantitative and semiquantitative determination of an antibody raised against it. Using S-layers as an immobilization matrix in comparison to amorphous polymers has advantages in that the closed monolayers of functional macromolecules on their outermost surface allows for strong signals in immunoassays, almost completely eliminates background and prevents diffusion.

```
=> s s layer protein
   6 FILES SEARCHED...
L22         1102 S LAYER PROTEIN

=> s l22 and (mature or crystalline or complete)
L23          304 L22 AND (MATURE OR CRYSTALLINE OR COMPLETE)

=> dup rem l23
PROCESSING COMPLETED FOR L23
L24          116 DUP REM L23 (188 DUPLICATES REMOVED)

=> s l24 and (express? or transform?)
   7 FILES SEARCHED...
L25          48 L24 AND (EXPRESS? OR TRANSFORM?)

=> d bib ab 1-48

L25  ANSWER 1 OF 48  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN   2002:236731  BIOSIS
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DN PREV200200236731
TI The surface layer (S-layer) glycoprotein of Geobacillus stearothermophilus NRS 2004/3a. Analysis of its glycosylation.
AU Schaeffer, Christina (1); Wugeditsch, Thomas; Kaehlig, Hanspeter; Scheberl, Andrea; Zayni, Sonja; Messner, Paul
CS (1) Zentrum fuer Ultrastrukturforschung, Ludwig Boltzmann-Institut fuer Molekulare Nanotechnologie, Universitaet fuer Bodenkultur Wien, Gregor-Mendel-Strasse 33, A-1180, Wien: crs@edv1.boku.ac.at Austria
SO Journal of Biological Chemistry, (February 22, 2002) Vol. 277, No. 8, pp. 6230-6239. http://www.jbc.org/. print.
ISSN: 0021-9258.
DT Article
LA English
AB Geobacillus stearothermophilus NRS 2004/3a possesses an oblique surface layer (S-layer) composed of glycoprotein subunits as the outermost component of its cell wall. In addition to the elucidation of the **complete** S-layer glycan primary structure and the determination of the glycosylation sites, the structural gene sgsE encoding the **S-layer protein** was isolated by polymerase chain

reaction-based techniques. The open reading frame codes for a protein of 903 amino acids, including a leader sequence of 30 amino acids. The **mature S-layer protein** has a calculated molecular mass of 93,684 Da and an isoelectric point of 6.1. Glycosylation of SgsE was investigated by means of chemical analyses, 600-MHz nuclear magnetic resonance spectroscopy, and matrix-assisted laser desorption ionization-time of flight mass spectrometry. Glycopeptides obtained after Pronase digestion revealed the glycan structure (fwdarw2)-alpha-L-Rhap-(1fwdarw3)-beta-L-Rhap-(1fwdarw2)-alpha-L-Rhap-(1fwdarw)n=13-18, with a 2-O-methyl group capping the terminal trisaccharide repeating unit at the non-reducing end of the glycan chains. The glycan chains are bound via the disaccharide core fwdarw3)-alpha-L-Rhap-(1fwdarw3)-alpha-L-Rhap-(1fwdarw and the linkage glycose beta-D-Galp in O-glycosidic linkages to the **S-layer protein** SgsE at positions threonine 620 and serine 794. This S-layer glycoprotein contains novel linkage regions and is the first one among eubacteria whose glycosylation sites have been characterized.

L25 ANSWER 2 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2002:146741 BIOSIS
DN PREV200200146741
TI Characterization of an S-layer glycoprotein produced in the course of S-layer variation of Bacillus stearothermophilus ATCC 12980 and sequencing and cloning of the sbsD gene encoding the protein moiety.
AU Egelseer, Eva M. (1); Danhorn, Thomas; Pleschberger, Magdalena; Hotzy, Christoph; Sleytr, Uwe B.; Sara, Margit
CS (1) Center for Ultrastructure Research, Ludwig Boltzmann-Institute for Molecular Nanotechnology, University of Agricultural Sciences, 1180, Vienna: egelseer@edv1.boku.ac.at Austria
SO Archives of Microbiology, (December, 2001) Vol. 177, No. 1, pp. 70-80. http://www.link.springer.de/link/service/journals/00203/index.htm. print. ISSN: 0302-8933.
DT Article
LA English
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered by an oblique lattice which consists of the **S-layer protein** SbsC. On SDS-polyacrylamide gels, the **mature S-layer protein** migrates as a single band with an apparent molecular mass of 122 kDa. During cultivation of B. stearothermophilus ATCC 12980 at 67degreeC instead of 55degreeC, a variant developed that had a secondary cell wall polymer identical to that of the wild-type strain, but it carried an S-layer glycoprotein that could be separated on SDS-polyacrylamide gels into four bands with apparent molecular masses of 92, 118, 150 and 175 kDa. After deglycosylation, only a single protein band with a molecular mass of 92 kDa remained. The **complete** nucleotide sequence encoding the protein moiety of this S-layer glycoprotein, termed SbsD, was established by PCR and inverse PCR. The sbsD gene of 2,709 bp is predicted to encode a protein of 96.2 kDa with a 30-amino-acid signal peptide. Within the 807 bp encoding the signal peptide and the N-terminal sequence (amino acids 31-269), different nucleotides for sbsD and sbsC were observed in 46 positions, but 70% of these mutations were silent, thus leading to a level of identity of 95% for the N-terminal parts. The level of identity of the remaining parts of SbsD and SbsC was below 10%, indicating that the lysine-, tyrosine- and arginine-rich N-terminal region in combination with a distinct type of secondary cell wall polymer remained conserved upon S-layer variation. The sbsD sequence encoding the **mature S-layer protein** cloned into the pET28a vector led to stable **expression** in Escherichia coli HMS174(DE3). This is the first example demonstrating that S-layer variation leads to the synthesis of an S-layer glycoprotein.

L25 ANSWER 3 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:473525 BIOSIS

DN PREV200100473525
TI Development of small high-copy-number plasmid vectors for gene **expression** in Caulobacter crescentus.
AU Umelo-Njaka, Elizabeth; Nomellini, John F.; Yim, Harry; Smit, John (1)
CS (1) Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3: jsmit@interchange.ubc.ca Canada
SO Plasmid, (July, 2001) Vol. 46, No. 1, pp. 37-46. print.
ISSN: 0147-619X.
DT Article
LA English
SL English
AB Caulobacter crescentus is a bacterium with a distinctive life cycle and so it is studied as a cell development model. In addition, we have adapted this bacterium for recombinant protein production and display based on the **crystalline** surface protein (S)-layer and its C-terminal secretion signal. We report here the development of small, high-copy-number plasmid vectors and methods for producing an obligate **expression** host.
The vectors are based on a narrow-host-range colE1-replicon-based plasmid commonly used in Escherichia coli, to which was added the replication origin of the IncQ plasmid RSF1010. C. crescentus strains were modified to enable plasmid replication by introduction of the RSF1010 repBAC genes at the recA locus. The small (4.0-4.5 kb) plasmids were in high copy numbers in both C. crescentus and E. coli and amenable to rapid methods for plasmid isolation and DNA sequencing. The method for introducing repBAC is suitable for other C. crescentus strains or any bacterium with an adequately homologous recA gene. Application of the vector for protein **expression**, based on the type I secretion system of the **S -layer protein**, when compared to constructs in broad-host-range plasmids, resulted in reduced time and steps required from clone construction to recombinant protein recovery and increased protein yield.

L25 ANSWER 4 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:289673 BIOSIS
DN PREV200100289673
TI Analysis of the structure-function relationship of the **S-layer protein** SbsC of Bacillus stearothermophilus ATCC 12980 by producing truncated forms.
AU Jarosch, Marina; Egelseer, Eva M.; Huber, Carina; Moll, Dieter; Mattanovich, Diethard; Sleytr, Uwe B.; Sara, Margit (1)
CS (1) Centre for Ultrastructure Research, Ludwig Boltzmann-Institute for Molecular Nanotechnology, University of Agricultural Sciences, 1180, Vienna: sara@edv1.boku.ac.at Austria
SO Microbiology (Reading), (May, 2001) Vol. 147, No. 5, pp. 1353-1363. print.
ISSN: 1350-0872.
DT Article
LA English
SL English
AB The **mature** surface layer (**S-layer**) **protein** SbsC of Bacillus stearothermophilus ATCC 12980 comprises amino acids 31-1099 and self-assembles into an oblique lattice type which functions as an adhesion site for a cell-associated high-molecular-mass exoamylase. To elucidate the structure-function relationship of distinct segments of SbsC, three N- and seven C-terminal truncations were produced in a heterologous **expression** system, isolated, purified and their properties compared with those of the recombinant **mature S-layer protein** rSbsC31-1099. With the various truncated forms it could be demonstrated that the N-terminal part (aa 31-257) is responsible for anchoring the S-layer subunits via a distinct type of secondary cell wall polymer to the rigid cell wall layer, but this positively charged segment is not required for the self-assembly of SbsC, nor for generating the oblique lattice structure. If present, the N-terminal part leads to the formation of in vitro double-layer self-assembly products. Affinity studies further showed that the

N-terminal part includes an exoamylase-binding site. Interestingly, the N-terminal part carries two sequences of 6 and 7 aa (AKAALD and KAAYEAA) that were also identified on the amylase-binding protein AbpA of Streptococcus gordonii. In contrast to the self-assembling N-terminal truncation rSbsC258-1099, two further N-terminal truncations (rSbsC343-1099, rSbsC447-1099) and three C-terminal truncations (rSbsC31-713, rSbsC31-844, rSbsC31-860) had lost the ability to self-assemble and stayed in the water-soluble state. Studies with the self-assembling C-terminal truncations rSbsC31-880, rSbsC31-900 and rSbsC31-920 revealed that the C-terminal 219 aa can be deleted without interfering with the self-assembly process, while the C-terminal 179 aa are not required for the formation of the oblique lattice structure.

L25 ANSWER 5 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:113721 BIOSIS
DN PREV200100113721
TI The **S-layer protein** of Lactobacillus acidophilus ATCC 4356: Identification and characterisation of domains responsible for S-protein assembly and cell wall binding.
AU Smit, Egbert; Oling, Frank; Demel, Rudy; Martinez, Beatriz; Pouwels, Peter H. (1)
CS (1) Department of Applied Microbiology and Gene Technology, TNO Nutrition and Food Research Institute, Utrechtseweg 48, 3700 AJ, Zeist: Pouwels@voeding.tno.nl Netherlands
SO Journal of Molecular Biology, (12 January, 2001) Vol. 305, No. 2, pp. 245-257. print.
ISSN: 0022-2836.
DT Article
LA English
SL English
AB Lactobacillus acidophilus, like many other bacteria, harbors a surface layer consisting of a protein (SA-protein) of 43 kDa. SA-protein could be readily extracted and crystallized in vitro into large **crystalline** patches on lipid monolayers with a net negative charge but not on lipids with a net neutral charge. Reconstruction of the S-layer from crystals grown on dioleoylphosphatidylserine indicated an oblique lattice with unit cell dimensions (a = 118 ANG, b = 53 ANG, and gamma = 102 degree) resembling those determined for the S-layer of Lactobacillus helveticus ATCC 12046. Sequence comparison of SA-protein with S-proteins from L. helveticus, Lactobacillus crispatus and the S-proteins encoded by the silent S-protein genes from L. acidophilus and L. crispatus suggested the presence of two domains, one comprising the N-terminal two-thirds (SAN), and another made up of the C-terminal one-third (SAC) of SA-protein. The sequence of the N-terminal domains is variable, while that of the C-terminal domain is highly conserved in the S-proteins of these organisms and contains a tandem repeat. Proteolytic digestion of SA-protein showed that SAN was protease-resistant, suggesting a compact structure. SAC was rapidly degraded by proteases and therefore probably has a more accessible structure. DNA sequence encoding SAN or Green Fluorescent Protein fused to SAC (GFP-SAC) were efficiently **expressed** in Escherichia coli. Purified SAN could crystallize into mono and multi-layered crystals with the same lattice parameters as those found for authentic SA-protein. A calculated SA-protein minus SAN density-difference map revealed the probable location, in projection, of the SAC domain, which is missing from the truncated SAN peptide. The GFP-SAC fusion product was shown to bind to the surface of L. acidophilus, L. helveticus and L. crispatus cells from which the S-layer had been removed, but not to non-stripped cells or to Lactobacillus casei.

L25 ANSWER 6 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:12819 BIOSIS
DN PREV200100012819
TI Characterization of the collagen-binding **S-layer protein** CbsA of Lactobacillus crispatus.

AU Sillanpaa, Jouko; Martinez, Beatriz; Antikainen, Jenni; Toba, Takahiro; Kalkkinen, Nisse; Tankka, Sanna; Lounatmaa, Kari; Keranen, Jaakko; Hook, Magnus; Westerlund-Wikstrom, Benita; Pouwels, Peter H.; Korhonen, Timo K. (1)

CS (1) Division of General Microbiology, Department of Biosciences, University of Helsinki, FIN 00014, Helsinki: timo.korhonen@helsinki.fi Finland

SO Journal of Bacteriology, (November, 2000) Vol. 182, No. 22, pp. 6440-6450. print.
ISSN: 0021-9193.

DT Article

LA English

SL English

AB The cbsA gene of Lactobacillus crispatus strain JCM 5810, encoding a protein that mediates adhesiveness to collagens, was characterized and **expressed** in Escherichia coli. The cbsA open reading frame encoded a signal sequence of 30 amino acids and a **mature** polypeptide of 410 amino acids with typical features of a bacterial **S-layer protein**. The cbsA gene product was **expressed** as a His tag fusion protein, purified by affinity chromatography, and shown to bind solubilized as well as immobilized type I and IV collagens. Three other Lactobacillus S-layer proteins, SlpA, CbsB, and SlpnB, bound collagens only weakly, and sequence comparisons of CbsA with these S-layer proteins were used to select sites in cbsA where deletions and mutations were introduced. In addition, hybrid S-layer proteins that contained the N or the C terminus from CbsA, SlpA, or SlpnB as well as N- and C-terminally truncated peptides from CbsA were constructed by gene fusion. Analysis of these molecules revealed the major collagen-binding region within the N-terminal 287 residues and a weaker type I collagen-binding region in the C terminus of the CbsA molecule. The mutated or hybrid CbsA molecules and peptides that failed to polymerize into a periodic S-layer did not bind collagens, suggesting that the crystal structure with a regular array is optimal for **expression** of collagen binding by CbsA. Strain JCM 5810 was found to contain another S-layer gene termed cbsB that was 44% identical in sequence to cbsA. RNA analysis showed that cbsA, but not cbsB, was transcribed under laboratory conditions. **S-layer-protein-expressing** cells of strain JCM 5810 adhered to collagen-containing regions in the chicken colon, suggesting that CbsA-mediated collagen binding represents a true tissue adherence property of L. crispatus.

L25 ANSWER 7 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 2000:85100 BIOSIS

DN PREV200000085100

TI **S-layer protein** production by Corynebacterium strains is dependent on the carbon source.

AU Soual-Hoebeke, Emmanuelle; de Sousa-D'Auria, Celia; Chami, Mohamed; Baucher, Maire-France; Guyonvarch, Armel; Bayan, Nicolas; Salim, Karima; Leblon, Gerard (1)

CS (1) Laboratoire de Biologie Moleculaire des Corynebacteries, Institut de Genetique et Microbiologie, UMR C8621 CNRS, Bat. 409, Universite Paris XI, 91405, Orsay Cedex France

SO Microbiology (Reading), (Dec., 1999) Vol. 145, No. 12, pp. 3399-3408.
ISSN: 1350-0872.

DT Article

LA English

SL English

AB Three strains of Corynebacterium producing various amounts of PS2 **S-layer protein** were studied. For all strains, more PS2 was produced if the bacteria were grown in minimal medium supplemented with lactate than if they were grown in minimal medium supplemented with glucose. The consumption of substrate and PS2 production was studied in cultures with mixed carbon sources. It was found that the inhibitory effect of glucose consumption was stronger than the stimulatory

effect of lactate in one strain, but not in the other two strains. The regulation of gene **expression** involved in S-layer formation may involve metabolic pathways, which probably differ between strains. S-layer organization was also studied by freeze-fracture electron microscopy. It was found that low levels of PS2 production correlated with the partial covering of the cell surface by a **crystalline** array. Finally, it was found that PS2 production was mainly regulated by changes in gene **expression** and that secretion was probably not a limiting step in PS2 accumulation.

L25 ANSWER 8 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:173092 BIOSIS
DN PREV199900173092
TI Self-assembly product formation of the Bacillus stearothermophilus PV72/p6 **S-layer protein** SbsA in the course of autolysis of Bacillus subtilis.
AU Howorka, Stefan; Sara, Margit; Lubitz, Werner; Kuen, Beatrix (1)
CS (1) Institut fur Mikrobiologie and Genetik, Universitat Wien, Dr. Bohrgasse 9, A-1030, Vienna Austria
SO FEMS Microbiology Letters, (March 15, 1999) Vol. 172, No. 2, pp. 187-196. ISSN: 0378-1097.
DT Article
LA English
AB In order to achieve high level **expression** and to study the release of a protein capable of self-assembly, the gene encoding the **crystalline** cell surface (**S-layer**) **protein** SbsA of Bacillus stearothermophilus PV72/p6, including its signal sequence, was cloned and **expressed** in Bacillus subtilis. To obtain high level **expression**, a tightly regulated, xylose-inducible, stably replicating multicopy-plasmid vector was constructed. After induction of **expression**, the **S-layer protein** made up about 15% of the total cellular protein content, which was comparable to the SbsA content of B. stearothermophilus PV72/p6 cells. During all growth stages, SbsA was poorly secreted to the ambient cellular environment by B. subtilis. Extraction of whole cells with guanidine hydrochloride showed that in late stationary growth phase cells 65% of the synthesised SbsA was retained in the peptidoglycan-containing layer, indicating that the rigid cell wall layer was a barrier for efficient SbsA secretion. Electron microscopic investigation revealed that SbsA release from the peptidoglycan-containing layer started in the late stationary growth phase at distinct sites at the cell surface leading to the formation of extracellular self-assembly products which did not adhere to the cell wall surface. In addition, intracellular sheet-like SbsA self-assembly products which followed the curvature of the cell became visible in partly lysed cells. Intracellularly formed self-assembly products remained intact even after **complete** lysis of the rigid cell envelope layer.

L25 ANSWER 9 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:473282 BIOSIS
DN PREV199800473282
TI The **S-layer protein** from Campylobacter rectus: Sequence determination and function of the recombinant protein.
AU Miyamoto, Manabu; Maeda, Hiroshi; Kitanaka, Michitaka; Kokeguchi, Susumu; Takashiba, Shogo; Murayama, Yuji (1)
CS (1) Dep. Periodontol., Okayama Univ. Dental Sch., 2-5-1 Shikati-cho Okayama 700-8525 Japan
SO FEMS Microbiology Letters, (Sept. 15, 1998) Vol. 166, No. 2, pp. 275-281. ISSN: 0378-1097.
DT Article
LA English
AB The gene encoding the **crystalline** surface layer (**S-layer**) **protein** from Campylobacter rectus, designated slp, was sequenced and the recombinant gene product was **expressed**

in Escherichia coli. The gene consisted of 4086 nucleotides encoding a protein with 1361 amino acids. The N-terminal amino acid sequence revealed that Slp did not contain a signal sequence, but that the initial methionine residue was processed. The deduced amino acid sequence displayed some common characteristic features of S-layer proteins previously reported. A homology search showed a high similarity to the Campylobacter fetus S-layer proteins, especially in their N-terminus. The C-terminal third of Slp exhibited homology with the RTX toxins from Gram-negative bacteria via the region including the glycine-rich repeats. The Slp protein had the same N-terminal sequence as a 104-kDa cytotoxin isolated from the culture supernatants of C. rectus. However, neither native nor recombinant Slp showed cytotoxicity against HL-60 cells or human peripheral white blood cells. These data support the idea that the N-terminus acts as an anchor to the cell surface components and that the C-terminus is involved in the assembly and/or transport of the protein.

L25 ANSWER 10 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1997:294708 BIOSIS
DN PREV199799593911
TI Oxygen-triggered synchronised variant formation of the S-layer carrying Bacillus stearothermophilus PV72 during continuous cultivation.
AU Schuster, Kurt C.; Pink, Tomas; Mayer, Harald F.; Hampel, Werner A.; Sara, Margit (1)
CS (1) Zentrum Ultrastrukforschung, Ludwig Boltzmann-Inst. Molekulare Nanotechnolgie, Univ. Bodenkultur, Gregor-Mendelstr. 33, 1180 Vienna Austria
SO Journal of Biotechnology, (1997) Vol. 54, No. 1, pp. 15-28. ISSN: 0168-1656.
DT Article
LA English
AB The **crystalline** cell surface layer (S-layer) of Bacillus stearothermophilus PV72 shows hexagonal lattice symmetry and is composed of a single protein species with a molecular weight of 130 000, termed SbsA. Stable synthesis of the SbsA on synthetic PVIII-medium was achieved during continuous cultivation of the wild-type strain at constant DO of 20%. When the DO was controlled at 50%, an apparent steady state was achieved after 2 volume exchanges after starting continuous culture. After 2-3 further volume exchanges, the respiratoric activity and culture fluorescence peaked markedly while the redox potential showed a steady increase. The increase in the optical density did not correlate with an increase in biomass concentration but rather reflected differences in the morphology of the cells and a slight sporulation. In addition to the physiological and morphological changes, a switch in **S-layer protein** synthesis was observed. Instead of the SbsA from the wild-type strain, an **S-layer protein** with a molecular weight of 97 000, termed SbsB, was produced which assembled into an oblique lattice type. The decrease in the wild-type **S-layer protein** content in biomass samples harvested during variant formation, freeze-etching and immunogold-labelling of whole cells confirmed that **expression** of SbsA was synchronously stopped in most, if not in all individual cells of the culture and was completely replaced by synthesis of the SbsB. Peptide mapping, N-terminal sequencing and Western blotting confirmed that both types of S-layer proteins were encoded by different genes. The hypothesis of oxygen-triggered synchronized variant formation was finally supported by computer simulations.

L25 ANSWER 11 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1996:219135 BIOSIS
DN PREV199698775264
TI **Expression** and purification of the **crystalline** surface layer protein of Rickettsia typhi.
AU Hahn, Myong-Joon (1); Chang, Woo-Hyun
CS (1) Dep. Microbiol., Coll. Med., Kon-Kuk Univ., Danwol-Dong, Choongju

380-701 South Korea
SO Microbiology and Immunology, (1996) Vol. 40, No. 3, pp. 233-236.
ISSN: 0385-5600.
DT Article
LA English
AB The **crystalline** surface layer (**S-layer**) **protein** (SLP) of Rickettsia typhi is known as the protective antigen against murine typhus. We previously reported a cloning and sequence analysis of the SLP gene of R. typhi (slpT) and showed that the open reading frame of this gene encodes both the SLP and a 32-kDa protein. To **express** only the SLP from this gene, the putative signal sequence and the 32-kDa protein portion were removed from the slpT. This protein was **expressed** in Escherichia coli as a fusion protein, consisting of the SLP and maltose binding protein. The recombinant protein reacted strongly with polyclonal antiserum of a patient with murine typhus.

L25 ANSWER 12 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1996:59818 BIOSIS
DN PREV199698631953
TI Identification, cloning, and nucleotide sequence of a silent **S-layer protein** gene of Lactobacillus acidophilus ATCC 4356 which has extensive similarity with the **S-layer protein** gene of this species.
AU Boot, Hein J. (1); Kolen, Carin P. A. M.; Pouwels, Peter H.
CS (1) BioCentrum Amsterdam, University Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam Netherlands
SO Journal of Bacteriology, (1995) Vol. 177, No. 24, pp. 7222-7230.
ISSN: 0021-9193.
DT Article
LA English
AB The bacterial S-layer forms a regular structure, composed of a monolayer of one (glyco)protein, on the surfaces of many prokaryotic species. S-layers are reported to fulfil different functions, such as attachment structures for extracellular enzymes and major virulence determinants for pathogenic species. Lactobacillus acidophilus ATCC 4356, which originates from the human pharynx, possesses such an S-layer. No function has yet been assigned to the S-layer of this species. Besides the structural gene (slpA) for the **S-layer protein** (S-protein) which constitutes this S-layer, we have identified a silent gene (slpB), which is almost identical to slpA in two regions. From the deduced amino acid sequence, it appears that the **mature** SB-protein (44,884 Da) is 53% similar to the S-A-protein (43,636 Da) in the N-terminal and middle parts of the proteins. The C-terminal parts of the two proteins are identical except for one amino acid residue. The physical properties of the deduced S-proteins are virtually the same. Northern (RNA) blot analysis shows that only the slpA gene is **expressed** in wild-type cells, in line with the results from sequencing and primer extension analyses, which reveal that only the slpA gene harbors a promoter, which is located immediately upstream of the region where the two genes are identical. The occurrence of in vivo chromosomal recombination between the two S-protein-encoding genes will be described elsewhere.

L25 ANSWER 13 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:391684 BIOSIS
DN PREV199598405984
TI A specific PulD homolog is required for the secretion of paracrystallin surface array subunits in Aeromonas hydrophila.
AU Thomas, Stephen R.; Trust, Trevor J. (1)
CS (1) Dep. Biochem. Microbiol. Univ. Victoria, Victoria, British Columbia V8W 3P6 Canada
SO Journal of Bacteriology, (1995) Vol. 177, No. 14, pp. 3932-3939.
ISSN: 0021-9193.
DT Article

LA English
AB Aeromonas hydrophila is an important pathogen of fish, and its high-virulence strains display a two-dimensional paracrystalline layer (S-layer) on their outermost surfaces. The nucleotide sequence of a 4.1-kb region located 700 hp upstream of the A. hydrophila TF7 **S-layer protein** gene (ahsA) has been determined. A sequence analysis of the region revealed the presence of three **complete** open reading frames ending in a gene encoding a 79.8-Kda polypeptide that shows high homology to the PulD family of secretion proteins. The sequenced region displays both organizational and sequence homology to the Xanthomonas campestris pv. campestris Xps secretory system. Insertional inactivation of the spsD (S-protein secretion D) gene showed that the loss of **expression** of the PulD homolog coincided with the localization of the S-protein in the periplasm and the loss of the S-layer from the surface of the bacterium. However, the secretion of the enzymes hemolysin, amylase, and protease was unaffected in the mutant with the nonfunctional spsD gene, as was the export of flagella and fimbrial proteins. Southern blot analysis showed that the spsD gene was not conserved among all strains of S-protein-producing A. hydrophila or Aeromonas veronii biotype sobria. Use of the promoterless chloramphenicol acetyltransferase gene showed that unlike pulD and its homologs, spsD contains its own promoter. A. hydrophila has been shown to contain the exe operon, which is responsible for the secretion of a number of extracellular enzymes in this bacterium. A fragment of DNA was generated from the exeD gene of A. hydrophila Ah65 by PCR and was subsequently used in hybridization studies to probe the chromosome of A. hydrophila TF7. The presence of an exeD homolog in A. hydrophila TF7 was found; therefore, the spsD gene encodes a second pulD homolog that displays a high specificity for the secretion of the S-protein. This gene appears to be part of a second terminal branch of the general secretory pathway in A. hydrophila.

L25 ANSWER 14 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:226712 BIOSIS
DN PREV199598241012
TI A lipopolysaccharide-binding domain of the Campylobacter fetus **S-layer protein** resides within the conserved N terminus of a family of silent and divergent homologs.
AU Dworkin, Joel; Tummuru, Murali K. R.; Blaser, Martin J. (1)
CS (1) Vanderbilt Univ. Sch. Med., Div. Infectious Diseases, A-3310 Medical Center North, Nasville, TN 37232-2605 USA
SO Journal of Bacteriology, (1995) Vol. 177, No. 7, pp. 1734-1741. ISSN: 0021-9193.
DT Article
LA English
AB Campylobacter fetus cells can produce multiple S-layer proteins ranging from 97 to 149 kDa, with a single form predominating in cultured cells. We have cloned, sequenced, and **expressed** in Escherichia coli a saPA homolog, sapA2, which encodes a full-length 1,109-amino-acid (112-kDa) **S-layer protein**. Comparison with the two previously cloned sapA homologs has demonstrated two regions of identity, approximately 70 hp before the open reading frame (ORF) and proceeding 550 hp into the ORF and immediately downstream of the ORF. The entire genome contains eight copies of each of these conserved regions. Southern analyses has demonstrated that sapA2 existed as a **complete** copy within the genome in all strains examined, although Northern (RNA) analysis has demonstrated that sapA2 was not **expressed** in the C. fetus strain from which it was cloned. Further Southern analyses revealed increasing sapA diversity as probes increasingly 3' within the ORF were used. Pulsed-field gel electrophoresis and then Southern blotting with the conserved N-terminal region of the sapA homologs as a probe showed that these genes were tightly clustered on the chromosome. Deletion mutagenesis revealed that the **S-layer protein** bound serospecifically to the C. fetus lipopolysaccharide via its conserved N-terminal region. These data indicated that the S-layer proteins shared

functional activity in the conserved N terminus but diverged in a semiconservative manner for the remainder of the molecule. Variation in **S-layer protein expression** may involve rearrangement of **complete** gene copies from a single large locus containing multiple sapA homologs.

L25 ANSWER 15 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:178656 BIOSIS
DN PREV199598192956
TI Tyrosine phosphorylation of the tetragonal paracrystalline array of Aeromonas hydrophila: Molecular cloning and high-level **expression** of the **S-layer protein** gene.
AU Thomas, Stephen R.; Trust, Trevor J. (1)
CS (1) Can. Bacterial Diseases Network Univ. Victoria, Victoria, BC V8W 3P6 Canada
SO Journal of Molecular Biology, (1995) Vol. 245, No. 5, pp. 568-581. ISSN: 0022-2836.
DT Article
LA English
AB High virulence strains of the fish pathogenic bacterium Aeromonas hydrophila produce a tetragonally arranged paracrystalline surface protein array (S-layer). The gene (ahsA) encoding the S-protein subunit of A. hydrophila TF7 was cloned into lambda-EMBL 3, and sub-cloned into pUC 18. **Transformation** into Escherichia coli led to stable high-levelexpression of full-size S-protein under the control of its native promoter. The DNA sequence revealed a 1406 base-pair open reading frame encoding a protein consisting of a 19 amino acid residue signal peptide, and a 448 residue 45,400 Da molecular mass **mature** protein with a predicted isoelectric point (pI) of 6.72 compared with the measured M-r of 52,000 and pI of 4.6. This suggested that the S-protein was post-translationally modified and in vivo cell labelling with (32P)orthophosphate, acid phosphatase digestion of S-protein, ascending thin-layer chromatography of partially acid hydrolysed S-protein and Western blot analysis with monoclonal anti-phosphotyrosine antibody showed that the S-protein of strain TF7 contained phosphotyrosine. S-proteins produced by the other strains of motile aeromonads tested also reacted with this anti-phosphotyrosine antibody. Cell fractionation studies employing plasmid-encoded ahsA showed that in A. hydrophila the S-protein subunits were secreted across the outer membrane by the native S-protein secretion pathway, while in E. coli and A. salmonicida the cloned A. hydrophila S-protein inserted into the outer membrane of the foreign host. These findings indicate that the process employed to translocate Aeromonas S-proteins across the outer membrane is highly specific.

L25 ANSWER 16 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:501476 BIOSIS
DN PREV199396125483
TI **S-layer protein** of Lactobacillus acidophilus ATCC4356: Purification, **expression** in Escherichia coli, and nucleotide sequence of the corresponding gene.
AU Boot, Hein J. (1); Kolen, Carin P. A. M.; Van Noort, Johannes M.; Pouwels, Peter H.
CS (1) Dep. Mol. Cell Biol., Sect. Mol. Biol., Univ. Amsterdam, Kruislaan 318, 109 SM Amsterdam Netherlands Antilles
SO Journal of Bacteriology, (1993) Vol. 175, No. 19, pp. 6089-6096. ISSN: 0021-9193.
DT Article
LA English
AB The cell surfaces of several Lactobacillus species are covered by a regular layer composed of a single species of protein, the S-protein. The 43-kDa S-protein of the neotype strain Lactobacillus acidophilus ATCC 4356, which originated from the pharynx of a human, was purified. Antibodies generated against purified S-protein were used to screen a lambda library containing chromosomal L. acidophilus ATCC 4356 DNA.

Several phages showing **expression** of this S-protein in Escherichia coli were isolated. A 4.0-kb DNA fragment of one of those phages hybridized to a probe derived from an internal tryptic fragment of the S-protein. The slpA gene, coding for the surface layer protein, was located entirely on the 4.0-kb fragment as shown by deletion analysis. The nucleotide sequence of the slpA gene was determined and appeared to encode a protein of 444 amino acids. The first 24 amino acids resembled a putative secretion signal, giving rise to a **mature** S-protein of 420 amino acids (44.2 kDa). The predicted isoelectric point of 9.4 is remarkably high for an S-protein but is in agreement with the data obtained during purification. The **expression** of the entire S-protein or of large, C-terminally truncated S-proteins is unstable in E. coli.

L25 ANSWER 17 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:433107 BIOSIS
DN PREV199396087732
TI Shift in **S-layer protein expression** responsible for antigenic variation in Campylobacter fetus.
AU Wang, Enze; Garcia, Manuel M.; Blake, Milan S.; Pei, Zhiheng; Blaser, Martin J. (1)
CS (1) Div. Infectious Dis., Dep. Med., Vanderbilt Univ Sch., Nashville, TN 37232 USA
SO Journal of Bacteriology, (1993) Vol. 175, No. 16, pp. 4979-4984. ISSN: 0021-9193.
DT Article
LA English
AB Campylobacter fetus strains possess regular paracrystalline surface layers (S-layers) composed of high-molecular-weight proteins and can change the size and **crystalline** structure of the predominant protein **expressed**. Polyclonal antisera demonstrate antigenic cross-reactivity among these proteins but suggest differences in epitopes. Monoclonal antibodies to the 97-kDa **S-layer protein** of Campylobacter fetus subsp. fetus strain 82-40LP showed three different reactivities. Monoclonal antibody 1D1 recognized 97-kDa S-layer proteins from all C. fetus strains studied; reactivity of monoclonal antibody 6E4 was similar except for epitopes in S-layer proteins from reptile strains and strains with type B lipopolysaccharide. Monoclonal antibody 2E11 only recognized epitopes on S-layer proteins from strains with type A lipopolysaccharide regardless of size. In vitro shift from a 97-kDa **S layer protein** to a 127-kDa **S-layer protein** resulted in different reactivity, indicating that size change was accompanied by antigenic variation. To examine in vivo variation, heifers were genitally challenged with Campylobacter fetus subsp. venerealis strains and the S-layer proteins from sequential isolates were characterized. Analysis with monoclonal antibodies showed that antigenic reactivities of the S-layer proteins were varied, indicating that these proteins represent a system for antigenic variation.

L25 ANSWER 18 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:432262 BIOSIS
DN PREV199396086887
TI Characterization of the cspB gene encoding PS2, an ordered surface-layer protein in Corynebacterium glutamicum.
AU Peyret, J. L.; Bayan, N.; Joliff, G.; Gulik-Krzywicki, T.; Mathieu, L.; Schechter, E.; Leblon, G. (1)
CS (1) Lab. Biol. Moleculaire Coryne-Bacteries, URA D1354 CNRS GDR 961, Univ. Paris-Sud, Bat. 409, 91405 Orsay Cedex France
SO Molecular Microbiology, (1993) Vol. 9, No. 1, pp. 97-109. ISSN: 0950-382X.
DT Article
LA English
AB PS2 is one of two major proteins detected in the culture media of various

Corynebacterium glutamicum strains. The coding and promoter regions of the cspB gene encoding PS2 were cloned in lambda gt11 using polyclonal antibodies raised against PS2 for screening. **Expression** of the cspB gene in Escherichia coli led to the production of a major anti-PS2 labelled peptide of 63 000 Da, corresponding presumably to the **mature** form of PS2. It was detected in the cytoplasm, periplasm and surrounding medium of E. coli. Three other slower migrating bands of 65 000, 68 000 and 72 000 Da were detected. The largest one probably corresponds to the precursor form of PS2 in E. coli. Analysis of the nucleotide sequence revealed an open reading frame (ORF) of 1533 nucleotides. The deduced 510-amino-acid polypeptide had a calculated molecular mass of 55 426 Da. According to the predicted amino acid sequence, PS2 is synthesized with a N-terminal segment of 30-amino-acid residues reminiscent of eukaryotic and prokaryotic signal peptides, and a hydrophobic domain of 21 residues near the C-terminus. Although no significant homologies were found with other proteins, it appears that some characteristics and the amino acid composition of PS2 share several common features with surface-layer proteins. The cspB gene was then disrupted in C. glutamicum by gene replacement. Freeze-etching electron microscopy performed on the wild-type strain indicated that the cell wall of C. glutamicum is covered with an ordered surface of proteins (surface layer, S-layer) which is in very close contact with other cell-wall components. These structures are absent from the cspB-disrupted strain but are present after reintroduction of the cspB gene on a plasmid into this mutant. Thus we demonstrate that the **S-layer protein** is the product of the cspB gene.

L25 ANSWER 19 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1990:27247 BIOSIS
DN BA89:14213
TI **S-LAYER PROTEIN** GENE OF ACETOGENIUM-KIVUI CLONING AND **EXPRESSION** IN ESCHERICHIA-COLI AND DETERMINATION OF THE NUCLEOTIDE SEQUENCE.
AU PETERS J; PETERS M; LOTTSPEICH F; BAUMEISTER W
CS MAX-PLANCK-INST. FUER BIOCHEMIE, 8033 MARTINSRIED, FRG.
SO J BACTERIOL, (1989) 171 (11), 6307-6315.
CODEN: JOBAAY. ISSN: 0021-9193.
FS BA; OLD
LA English
AB Acetogenium kivui is an anaerobically growing thermophilic bacterium with a gram-positive type of cell wall structure. The outer surface is covered with a hexagonally packed surface (S) layer. The gene coding for the S-layer polypeptide was cloned in Escherichia coli on two overlapping fragments by using the plasmid pUC18 as the vector. It was **expressed** under control of a cloned Acetogenium promoter or the lacZ gene. We determined the **complete** sequence of the structural gene. The **mature** polypeptide comprises 736 amino acids and is preceded by a typical procaryotic signal sequence of 26 amino acids. It is weakly acidic, weakly hydrophilic, and contains a relatively high proportion of hydroxyamino acids, including two clusters of serine and threonine residues. An N-terminal region of about 200 residues is homologous to the N-terminal part of the middle wall protein, one of the two S-layer proteins of Bacillus brevis, and there is also an internal homology within the N-terminal region of the A. kivui polypeptide.

L25 ANSWER 20 OF 48 MEDLINE
AN 1998195731 MEDLINE
DN 98195731 PubMed ID: 9534241
TI The S-layer gene of Lactobacillus helveticus CNRZ 892: cloning, sequence and heterologous **expression**.
AU Callegari M L; Riboli B; Sanders J W; Cocconcelli P S; Kok J; Venema G; Morelli L
CS Istituto di Microbiologia, Piacenza, Italy.. mcallega@cr.unicatt.it
SO MICROBIOLOGY, (1998 Mar) 144 (Pt 3) 719-26.

Journal code: BXW; 9430468. ISSN: 1350-0872.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AJ001931; GENBANK-X91199
EM 199805
ED Entered STN: 19980520
Last Updated on STN: 20000303
Entered Medline: 19980512
AB Lactobacillus helveticus CNRZ 892 contains a surface layer (S-layer) composed of protein monomers of 43 kDa organized in regular arrays. The gene encoding this protein (slpH) has been cloned in Escherichia coli and sequenced. slpH consists of 440 codons and is preceded by a ribosome-binding site (RBS) and followed by a putative rho-independent terminator. Indeed, Northern analysis revealed that slpH is a monocistronic gene. The gene is preceded by a possible promotor of which the -35 and -10 hexanucleotides are separated by 17 nt. By primer extension analysis the transcription start site was mapped at 7 nt downstream of the -10 sequence while the deduced amino acid sequence of SlpH shows a leader peptide of 30 aa. The slpH gene has been amplified by PCR and the fragment, carrying the **complete** gene from the RBS to the stop codon, has been cloned in a lactococcal gene **expression** vector downstream of promoter P32. Lactococcus lactis MG1363 carrying the resulting plasmid produced and secreted an S-layer monomer with the same molecular mass as the authentic L. helveticus CNRZ 892 SlpH, as judged by SDS-PAGE. Immunoelectron microscopy revealed that SlpH was bound to the lactococcal cell walls in small clumps and accumulated in the growth medium as small sheets.

L25 ANSWER 21 OF 48 MEDLINE
AN 97422863 MEDLINE
DN 97422863 PubMed ID: 9276928
TI Molecular biology of S-layers.
AU Bahl H; Scholz H; Bayan N; Chami M; Leblon G; Gulik-Krzywicki T; Shechter E; Fouet A; Mesnage S; Tosi-Couture E; Gounon P; Mock M; Conway de Macario E; Macario A J; Fernandez-Herrero L A; Olabarria G; Berenguer J; Blaser M J; Kuen B; Lubitz W; Sara M; Pouwels P H; Kolen C P; Boot H J; Resch S
CS Universitat Rostock, Germany.
NC RO1 24145
SO FEMS MICROBIOLOGY REVIEWS, (1997 Jun) 20 (1-2) 47-98. Ref: 197
Journal code: AO4; 8902526. ISSN: 0168-6445.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LA English
FS Priority Journals
OS GENBANK-U38842; GENBANK-X91199; GENBANK-X92752
EM 199710
ED Entered STN: 19971013
Last Updated on STN: 19971013
Entered Medline: 19971001
AB In this chapter we report on the molecular biology of **crystalline** surface layers of different bacterial groups. The limited information indicates that there are many variations on a common theme. Sequence variety, antigenic diversity, gene **expression**, rearrangements, influence of environmental factors and applied aspects are addressed. There is considerable variety in the S-layer composition, which was elucidated by sequence analysis of the corresponding genes. In Corynebacterium glutamicum one major cell wall protein is responsible for the formation of a highly ordered, hexagonal array. In contrast, two abundant surface proteins from the S-layer of Bacillus anthracis. Each protein possesses three S-layer homology motifs and one protein could be a

virulence factor. The antigenic diversity and ABC transporters are important features, which have been studied in methanogenic archaea. The **expression** of the S-layer components is controlled by three genes in the case of Thermus thermophilus. One has repressor activity on the S-layer gene promoter, the second codes for the **S-layer protein**. The rearrangement by reciprocal recombination was investigated in Campylobacter fetus. 7-8 S-layer proteins with a high degree of homology at the 5' and 3' ends were found. Environmental changes influence the surface properties of Bacillus stearothermophilus. Depending on oxygen supply, this species produces different S-layer proteins. Finally, the molecular bases for some applications are discussed. Recombinant S-layer fusion proteins have been designed for biotechnology.

L25 ANSWER 22 OF 48 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 2002013097 EMBASE
TI Characterization of an S-layer glycoprotein produced in the course of S-layer variation of Bacillus stearothermophilus ATCC 12980 and sequencing and cloning of the sbsD gene encoding the protein moiety.
AU Egelseer E.M.; Danhorn T.; Pleschberger M.; Hotzy C.; Sleytr U.B.; Sara M.
CS E.M. Egelseer, Center Ultrastructure Research, Ludwig Boltzmann-Ins. Molec. Nanot., University of Agricultural Sciences, 1180 Vienna, Austria. egelseer@edv1.boku.ac.at
SO Archives of Microbiology, (2002) 177/1 (70-80).
Refs: 39
ISSN: 0302-8933 CODEN: AMICCW
CY Germany
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered by an oblique lattice which consists of the **S-layer protein** SbsC. On SDS-polyacrylamide gels, the **mature S-layer protein** migrates as a single band with an apparent molecular mass of 122 kDa. During cultivation of B. stearothermophilus ATCC 12980 at 67.degree.C instead of 55.degree.C, a variant developed that had a secondary cell wall polymer identical to that of the wild-type strain, but it carried an S-layer glycoprotein that could be separated on SDS-polyacrylamide gels into four bands with apparent molecular masses of 92, 118, 150 and 175 kDa. After deglycosylation, only a single protein band with a molecular mass of 92 kDa remained. The **complete** nucleotide sequence encoding the protein moiety of this S-layer glycoprotein, termed SbsD, was established by PCR and inverse PCR. The sbsD gene of 2,709 bp is predicted to encode a protein of 96.2 kDa with a 30-amino-acid signal peptide. Within the 807 bp encoding the signal peptide and the N-terminal sequence (amino acids 31-269), different nucleotides for sbsD and sbsC were observed in 46 positions, but 70% of these mutations were silent, thus leading to a level of identity of 95% for the N-terminal parts. The level of identity of the remaining parts of SbsD and SbsC was below 10%, indicating that the lysine-, tyrosine- and arginine-rich N-terminal region in combination with a distinct type of secondary cell wall polymer remained conserved upon S-layer variation. The sbsD sequence encoding the **mature S-layer protein** cloned into the pET28a vector led to stable **expression** in Escherichia coli HMS174(DE3). This is the first example demonstrating that S-layer variation leads to the synthesis of an S-layer glycoprotein.

L25 ANSWER 23 OF 48 WPIDS (C) 2002 THOMSON DERWENT
AN 1996-171046 [17] WPIDS
CR 1994-066249 [09]
DNC C1996-053926
TI **Expression** and presentation of polypeptide heterologous to Caulobacter **S-layer protein** to bacterium's

environment - by cloning in-frame into Caulobacter rsaA gene, and **expressing** as fusion prod. with **S-layer protein**.

DC B04 C06 D16 F09
IN BINGLE, W H; SMIT, J
PA (UYBR-N) UNIV BRITISH COLUMBIA
CYC 1
PI US 5500353 A 19960319 (199617)* 22p
ADT US 5500353 A CIP of US 1992-895367 19920609, US 1994-194290 19940209
PRAI US 1994-194290 19940209; US 1992-895367 19920609
AB US 5500353 A UPAB: 19960428

Expressing and presenting to the environment of a Caulobacter, a polypeptide heterologous to a Caulobacter **S-layer protein**, comprises cloning a sequence encoding a heterologous polypeptide in-frame into a Caulobacter rsaA gene, where the polypeptide is: (a) **expressed** and presented to the environment of the Caulobacter as a Caulobacter **S-layer protein** fusion prod.; and (b) lacks the capacity to form a disulphide bond within the fusion prod. when **expressed** by the Caulobacter.

USE - The method is used to present vaccine candidate epitopes at the cell surface, e.g. in outdoor freshwater environments and in fish vaccines. It may also be used to grow Caulobacter in wood pulp suspensions at an appropriate time in the wood pulping process, to provide enzymatic decomposition of the wood pulp structure, and therefore permit more effective penetration of bleaching agents, so reducing the use of chlorine-based bleaching agents.

ADVANTAGE - The **S-layer protein** is synthesised in large quantities and has a generally repetitive sequence. This allows development of systems for synthesis of a relatively large amts. of heterologous material as a fusion prod, with an **S-layer protein**. The method is partic. suitable for use in a bioreactor system. The 2D, **crystalline** array of the S-protein layer has a geometrically regular, repetitive structure which provides an ideal means for dense packing and presentation of a foreign epitope to an immune system.

Dwg.0/9

L25 ANSWER 24 OF 48 WPIDS (C) 2002 THOMSON DERWENT
AN 1994-066249 [09] WPIDS
CR 1996-171046 [17]
DNC C1994-029749
TI Prodn. of heterologous polypeptides in bacteria, partic. Caulobacter - by **expression** of a fusion prod. of the polypeptide sequence and a bacterial **S-layer protein** gene.
DC B04 C06 D15 D16
IN BINGLE, W H; SMIT, J
PA (UYBR-N) UNIV BRITISH COLUMBIA
CYC 1
PI CA 2090549 A 19931210 (199409)* 27p
ADT CA 2090549 A CA 1993-2090549 19930226
PRAI US 1992-895367 19920609
AB CA 2090549 A UPAB: 19960503

A novel bacterium has an S-layer, where the bacterium's **S-layer protein** gene contains one or more sequences encoding one or more functional heterologous polypeptides and the S-layer is a fusion prod. of the **S-layer protein** and the heterologous polypeptides. The heterologous polypeptide may be e.g. cellulase, xylanase or a metallothionein.

USE/ADVANTAGE - The **S-layer protein** bacterial system can be used in bioreactors, e.g. to bind toxic metals in sewage, waste water etc., or for treatment of wood pulp suspensions. The system can also be used to produce heterologous proteins at the organisms cell surface for use in vaccines, partic. fish vaccines.

The **S-layer protein** is synthesised in

large quantities and has a generally repetitive sequence, permitting the synthesis of large amts. of heterologous protein as a fusion prod. and presentation at the cell surface.

In an example, the rsa A (S-layer) gene of C-crescentus was provided as pTZ188U:ras A delta P. Using Taq I, a partial digestion of the rsaA gene in pTZ18U:rsa A delta P produced a gp. of linearised segments with random Taq I sites cleaved. The linearised segments were modified by use of a tagged linker nutagenesis procedure using a 126p BamHI linker carried in puc102k.

Those prods. that produced a full-length protein in E.coli were transferred to pWB1 and introduced into C.crescentus. The **transformants** were analysed for the ability to produce a full-length protein in C.crescentus and to produce the **crystalline** S-layer on their surface. A Taq I site corresponding to amino acid 188 was identified. The coding sequence of monkey metallothionein II peptide was introduced into this site and **expressed** to produce a prod. which bound cadmium and copper.

Dwg.0/6

Dwg.0/6

L25 ANSWER 25 OF 48 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN 2001-11881 BIOTECHDS
TI Development of small high-copy-number plasmid-vectors for gene **expression** in Caulobacter crescentus;
plasmid pHY119Cm and plasmid pHY119Km construction with application in gene **expression** in Caulobacter crescentus with the resultant recombinant protein displayed by surface display
AU Umelo-Najaka E; Nomellini J F; Yim H; *Smit J
CS Univ.British-Columbia
LO Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada.
Email: jsmit@interchange.ubc.ca
SO Plasmid; (2001) 46, 1, 37-46
CODEN: PLSMDX ISSN: 0147-619X
DT Journal
LA English
AB Caulobacter crescentus was adapted as a host cell for recombinant protein production and display. Adaptation involved the **crystalline** surface protein (S)-layer and its C-terminal secretion signal. The development of small high-copy-number (HCN) plasmid vectors and methods for producing an obligate **expression** host are described. The vectors are based on a narrow-host-range colE1-replicon-based plasmid used in Escherichia coli, to which was added the replication origin of the IncQ plasmid RSF1010. C. crescentus strains were modified to enable plasmid replication by introduction of the RSF1010 repBAC genes at the recA locus. The 4.0-4.5 kb plasmids were in HCN in C. crescentus and E. coli and amenable to rapid plasmid isolation and sequencing. The method for introducing repBAC is suited to other C. crescentus strains or any bacterium with a homologous recA gene. Application of the vector for protein **expression** based on type I secretion system of the **S-layer protein**, when compared to constructs in broad-host range plasmids, gave reduced time and steps needed from clone construction to recombinant protein recovery and increased protein yield. (25 ref)

L25 ANSWER 26 OF 48 LIFESCI COPYRIGHT 2002 CSA
AN 1998:14212 LIFESCI
TI IV. Molecular biology of S-layers
AU Bahl, H.; Scholz, H.; Bayan, N.*; Chami, M.; Leblon, G.; Gulik-Krzywicki, T.; Shechter, E.; Fouet, A.; Mesnage, S.; Tosi-Couture, E.; Gounon, P.; Mock, M.; De Macario, E.C.; Macario, A.J.L.; Fernandez-Herrero, L.A.; et al.
CS Lab. des Biomembranes, URA 1116 CNRS, Univ. de Paris-Sud, F-91405 Orsay, France

SO pp. 47-98.
Meeting Info.: International workshop structure, biochemistry, molecular biology and applications of microbial S-layers. Rothenburg o.d. Tauber, Germany. Sep 1996.
DT Book
TC Conference; General Review
FS J
LA English
SL English
AB In this chapter we report on the molecular biology of **crystalline** surface layers of different bacterial groups. The limited information indicates that there are many variations on a common theme. Sequence variety, antigenic diversity, gene **expression**, rearrangements, influence of environmental factors and applied aspects are addressed. There is considerable variety in the S-layer composition, which was elucidated by sequence analysis of the corresponding genes. In Corynebacterium glutamicum one major cell wall protein is responsible for the formation of a highly ordered, hexagonal array. In contrast, two abundant surface proteins form the S-layer of Bacillus anthracis. Each protein possesses three S-layer homology motifs and one protein could be a virulence factor. The antigenic diversity and ABC transporters are important features, which have been studied in methanogenic archaea. The **expression** of the S-layer components is controlled by three genes in the case of Thermus thermophilus. One has repressor activity on the S-layer gene promoter, the second codes for the **S-layer protein**. The rearrangement by reciprocal recombination was investigated in Campylobacter fetus. 7-8 S-layer proteins with a high degree of homology at the 5' and 3' ends were found. Environmental changes influence the surface properties of Bacillus stearothermophilus. Depending on oxygen supply, this species produces different S-layer proteins. Finally, the molecular bases for some applications are discussed. Recombinant S-layer fusion proteins have been designed for biotechnology.

L25 ANSWER 27 OF 48 CAPLUS COPYRIGHT 2002 ACS
AN 2001:454368 CAPLUS
DN 135:207087
TI Molecular characterization of the surface layer proteins from Clostridium difficile
AU Calabi, Emanuela; Ward, Steven; Wren, Brendan; Paxton, Thanai; Panico, Maria; Morris, Howard; Dell, Anne; Dougan, Gordon; Fairweather, Neil
CS Department of Biology and Biochemistry, Imperial College, London, SW7 2AY, UK
SO Molecular Microbiology (2001), 40(5), 1187-1199
CODEN: MOMIEE; ISSN: 0950-382X
PB Blackwell Science Ltd.
DT Journal
LA English
AB Many bacteria **express** a surface-exposed proteinaceous layer, termed the S-layer, which forms a regular two-dimensional array visible by electron microscopy. Clostridium difficile is unusual in **expressing** two S-layer proteins (SLPs), which are of varying size in a no. of strains. In an approach combining mol. biol. with mass spectrometric sequencing strategies, the authors have identified the structural gene (slpA) for the S-layer from three strains of C. difficile. Both proteins are derived from a common precursor, and processing involves the removal of a signal peptide and a second cleavage to release the two **mature** SLPs. To the authors' knowledge, this is the first example in which two SLPs have been shown to derive from a single gene product through post-translational processing, rather than from the **expression** of sep. genes. The higher mol. wt. (MW) SLP is highly conserved among the three strains, whereas the lower MW SLP shows considerable sequence diversity, reflecting the results from Western blotting. The high-MW SLP shows weak homol. to N-acetyl muramoyl-L-alanine amidase from Bacillus subtilis, and both the native SLP

from C. difficile and a recombinant protein **expressed** in Escherichia coli were found to display amidase activity by zymog. The high-MW SLPs showed evidence of glycosylation, whereas the lower MW proteins did not. A family of genes with sequence homol. to the amidase domain of the high-MW SLP was identified in the C. difficile strain 630 genome, some of which are located in the same region of the genome as slpA and were shown by reverse transcription-polymerase chain reaction (RT-PCR) anal. to be transcribed.

RE.CNT 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L25 ANSWER 28 OF 48 CAPLUS COPYRIGHT 2002 ACS
AN 1996:579350 CAPLUS
DN 125:239776
TI The **complete** nucleotide sequence of the Bacillus licheniformis NM105 S-layer-encoding gene
AU Zhu, Xiaorong; McVeigh, Richard R.
CS Department of Physiology, Biophysics, UMDNJ - Robert Wood Johnson Medical School, Piscataway, NJ, 08854-5635, USA
SO Gene (1996), 173(2), 189-194
CODEN: GENED6; ISSN: 0378-1119
DT Journal
LA English
AB A protein present on the cell surface of Bacillus licheniformis (Bl) NM105 was identified as an S-layer (OlpA in this paper), a protein present on many bacterial cell surfaces. Purifn., SDS-PAGE and isoelectrofocusing showed one 94-kDa, slightly acidic (pI 6.5) protein band (defined as OlpA). The pure protein OlpA, has a tetragonal symmetry of its morphol. subunits. Following Edman degrdn., three 17-mer oligodeoxyribonucleotide (oligo) probes corresponding to the N-terminal sequence of OlpA were synthesized and used for gene cloning. The nucleotide (nt) sequence of the cloned gene (olpA) showed an ORF and encoded an 874 amino acid (aa) protein. In the promoter region of olpA, there appear to be -10 and -35 .sigma.A-binding sites, as well as -10 and -35 regions specific for .sigma.H. The existence of these two potential promoters suggests that OlpA would be produced during both the vegetative and sporulating stages of growth. The ribosome-binding site (RBS) sequence perfectly matched its consensus sequence, suggesting a high efficiency of translation of olpA. A typical 29-aa leader peptide, characteristic of secretory proteins in Bacilli, is present in the OlpA pre-protein sequence. In olpA, there are two stem-loop structures in tandem, downstream from the stop codon. These stem-loops are probably involved in prolonged olpA **expression**, by extending the half life of the mRNA.

L25 ANSWER 29 OF 48 CAPLUS COPYRIGHT 2002 ACS
AN 1993:487739 CAPLUS
DN 119:87739
TI Sequence of the S-layer gene of Thermus thermophilus HB8 and functionality of its promoter in Escherichia coli
AU Faraldo, M. M.; De Pedro, M. A.; Berenguer, J.
CS Cent. Biol. Mol., Univ. Auton. Madrid, Madrid, 28049, Spain
SO J. Bacteriol. (1992), 174(22), 7458-62
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB The nucleotide sequence of the slpA gene, which is responsible for the synthesis of the **S-layer protein** of T. thermophilus HB8, is described. This gene is transcribed as a unit in which the coding region is preceded by a 127-base-long leader mRNA sequence. The promoter region is also recognized by the RNA polymerase of E. coli because of the presence of homologous -35 and -10 boxes. Homol. with other promoters from Thermus spp. are also presented.

L25 ANSWER 30 OF 48 CAPLUS COPYRIGHT 2002 ACS

AN 1991:18201 CAPLUS
DN 114:18201
TI Surface array protein of Campylobacter fetus. Cloning and gene structure
AU Blaser, Martin J.; Gotschlich, Emil C.
CS Lab. Bacteriol. Immunol., Rockefeller Univ., New York, NY, 10021, USA
SO J. Biol. Chem. (1990), 265(24), 14529-35
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB The high mol. mass (97-149 kDa) surface array proteins (SAP) of C. fetus are crit. to virulence. A bank of 160,000 random 1.0-6.5-kilobase (kb) chromoosmal DNA fragments of C. fetus strain 84-32 (23D) was created using .lambda.gt11. Screening this bank in Escherichia coli Y1090 with antibody raised against purified SAP permitted isolation and purifn. of a clone with a 4.0-kb insert. Subcloning this insert in the E. coli vector, pUC9, permitted **expression** of a protein of .apprxeq.100 kDa, not fused with .beta.-galactosidase or inducible by isopropyl-.beta.-D-thiogalactopyranoside. Digestion with restriction endonucleases and construction of deletion mutations indicated that the gene extended over 2.8 kb, proceeding toward the start of the .beta.-galactosidase gene. Taking advantage of a unique PstI site at 1.7 kb, the authors subcloned PstI-EcoRI fragments in both orientations into M13 vectors, then generated and sequenced 48 deletion mutants. In the 3974-base insert, an open reading frame, beginning at nucleotide 24 and terminating at 2825, was found to encode a 933-amino acid polypeptide having a calcd. mol. mass of 96,758 daltons. The first 20 amino acids exactly match those detd. from amino-terminal sequencing, indicating that this protein is secreted without a leader sequence. The deduced amino acid compn. matches that of the purified SAP. A ribosomal binding site was identified 9 bases upstream and a putative transcription terminator 21 bases downstream. There is partial homol. of primary and secondary structure with 5 other bacterial S-layer proteins.

L25 ANSWER 31 OF 48 CAPLUS COPYRIGHT 2002 ACS
AN 1990:113022 CAPLUS
DN 112:113022
TI Cloning and sequencing of the gene encoding a 125-kilodalton surface-layer protein from Bacillus sphaericus 2362 and of a related cryptic gene
AU Bowditch, Ron D.; Baumann, Paul; Yousten, Allan A.
CS Dep. Microbiol., Univ. California, Davis, CA, 95616, USA
SO J. Bacteriol. (1989), 171(8), 4178-88
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB Using the vector pGEM-4-blue, a 4251-base-pair DNA fragment contg. the gene for the surface (**S**)-**layer protein** of B. sphaericus 2362 was cloned into Escherichia coli. Detn. of the nucleotide sequence indicated an open reading frame (ORF) coding for a protein of 1176 amino acids with a mol. size of 125 kilodaltons (kDa). A protein of this size which reacted with antibody to the 122-kDa **S-layer protein** of B. sphaericus was detected in cells of E. coli contg. the recombinant plasmid. Anal. of the deduced amino acid sequence indicated a highly hydrophobic N-terminal region which had the characteristics of a leader peptide. The first amino acid of the N-terminal sequence of the 122-kDa **S-layer protein** followed the predicted cleavage site of the leader peptide in the 125-kDa protein. A sequence characteristic of promoters **expressed** during vegetative growth was found within a 177-base-pair region upstream from the ORF coding for the 125-kDa protein. This putative promoter may account for the **expression** of this gene during the vegetative growth of B. sphaericus and E. coli. The gene for the 125-kDa protein was followed by an inverted repeat characteristic of teminators. Downstream from this gene (11.2 kilobases) was an ORF coding for a putative 80-kDa protein having a high sequence similarity to

the 125-kDa protein. Evidence was presented indicating that this gene is cryptic.

L25 ANSWER 32 OF 48 USPATFULL
AN 2002:92298 USPATFULL
TI **EXPRESSION** OF SURFACE LAYER PROTEINS
IN DEBLAERE, ROLF Y., WAARSCHOOT, BELGIUM
DESOMER, JAN, DRONGEN, BELGIUM
DHAESE, PATRICK, DRONGEN, BELGIUM
PA MCDERMOTT, WILL, EMERY (non-U.S. corporation)
PI US 2002048816 A1 20020425
AI US 1998-137531 A1 19980821 (9)
RLI Division of Ser. No. US 1996-682517, filed on 17 Sep 1996, GRANTED, Pat. No. US 5874267
PRAI WO 1995-EP147 19950113
GB 1994-650 19940114
DT Utility
FS APPLICATION
LREP MCDERMOTT WILL & EMERY, 600 13TH STREET, N.W., WASHINGTON, DC, 20005-3096
CLMN Number of Claims: 56
ECL Exemplary Claim: 1
DRWN 36 Drawing Page(s)
LN.CNT 3325
AB A host cell which is provided with a S-layer comprising a fusion polypeptide consisting essentially of:

(a) at least sufficient of a **S-layer protein** for a S-layer composed thereof to assemble, and

(b) a heterologous polypeptide which is fused to either the carboxy terminus of (a) or the amino terminus of (a) and which is thereby presented on the outer surface of the said cell; can be used as a vaccine, for screening for proteins and antigens and as a support for immobilizing an enzyme, peptide or antigen.

L25 ANSWER 33 OF 48 USPATFULL
AN 2002:48024 USPATFULL
TI NOVEL VACCINES AND PHARMACEUTICAL COMPOSITIONS USING MEMBRANE VESICLES OF MICROORGANISMS, AND METHODS FOR PREPARING SAME
IN KADURUGAMUWA, JAGATH L., GUELPH, CANADA
BEVERIDGE, TERRY J., ELORA, CANADA
PI US 2002028215 A1 20020307
AI US 1999-370860 A1 19990809 (9)
DT Utility
FS APPLICATION
LREP DOUGLAS P MUELLER, MERCHANT & GOULD PC, 3100 NORWEST CENTER, 90 SOUTH SEVENTH STREET, MINNEAPOLIS, MN, 55402
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN 35 Drawing Page(s)
LN.CNT 2647
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to novel vaccines and pharmaceutical compositions using membrane vesicles of microorganisms, methods for preparing same, and their use in the prevention and treatment of infectious diseases.

L25 ANSWER 34 OF 48 USPATFULL
AN 2002:16912 USPATFULL
TI **EXPRESSION** AND SECRETION OF HETEROLOGOUS POLYPEPTIDES FROM CAULOBACTER
IN SMIT, JOHN, RICHMOND, CANADA
BINGLE, WADE H., VANCOUVER, CANADA
NOMELLINI, JOHN F., RICHMOND, CANADA

PI US 2002009792 A1 20020124
AI US 1999-379931 A1 19990824 (9)
RLI Continuation of Ser. No. US 1996-614377, filed on 12 Mar 1996, GRANTED, Pat. No. US 5976864 Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, GRANTED, Pat. No. US 5500353 Continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, ABANDONED
DT Utility
FS APPLICATION
LREP Y ROCKY TSAO, FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 021102804
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 11 Drawing Page(s)
LN.CNT 1124
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB DNA constructs are provided which code for at least the extreme C-terminal amino acids of the rsaA protein of Caulobacter crescentus fused with heterologous polypeptides. Baterial cells containing, or which **express** the DNA constructs and secrete the resulting protein are also provided. Chimeric proteins including the C-terminal amino acids of the rsaA protein are provided, including chimeric proteins comprising antigenic epitopes of the Infectious Hematopoietic Necrosis Virus.

L25 ANSWER 35 OF 48 USPATFULL
AN 2001:167594 USPATFULL
TI Method of producing a structured layer
IN Sleytr, Uwe B., Vienna, Austria
Pum, Dietmar, Vienna, Austria
Loschner, Hans, Vienna, Austria
PA IMS-Ionen Mikrofabrikations Systems GmbH, Vienna, Austria (non-U.S. corporation)
PI US 6296700 B1 20011002
AI US 1999-388289 19990901 (9)
RLI Continuation-in-part of Ser. No. WO 1998-AT50, filed on 4 Mar 1998
PRAI AT 1997-373 19970304
DT Utility
FS GRANTED
EXNAM Primary Examiner: Kunemund, Robert
LREP Vigil, Thomas R.
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 15 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 387
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to a method for producing a structured layer of defined functional molecules on the surface of a flat substrate, on the surface of which structures having different surface properties, at least as regards their hydrophobicity, are produced. A monolayer of a protein-containing **crystalline** cell surface layer (S layer) is deposited by recrystallization on said structured surface. Said S Layer binds only to those structured areas of the surface characterized by raised hydrophobicity. Alternatively, a structured S-layer may also be produced on the basis of a monolayer of an S layer deposited on a substrate by irradiating predefined sections of said layer to be structured with radiation of a predetermined intensity and energy. In the irradiated sections of the S-layer this suppresses the binding or intercalating ability of at least one surface. Functional molecules can bind to or become intercalated in the surface or the intermediate spaces of the crystal lattice, for example for use as bio-sensors.

L25 ANSWER 36 OF 48 USPATFULL
AN 2001:144913 USPATFULL
TI NOVEL ADHERENCE FACTORS OF NON PATHOGENIC MICROORGANISMS AND

APPLICATIONS THEREOF FOR SCREENING MICROORGANISMS FOR SPECIFIC PROBIOTIC PROPERTIES; NOVEL PHARMACEUTICAL COMPOSITIONS AND FOOD ADDITIVES COMPRISING SUCH MICROORGANISMS AND ADHERENCE FACTORS

IN LEER, ROBERT JAN, VOORBURG, Netherlands
POUWELS, PIETER HENDRIK, RIJSWIJK, Netherlands
CONWAY, PATRICIA LYNNE, LE PEROUSE NSW, Australia

PI US 2001018048 A1 20010830
AI US 1998-51755 A1 19980930 (9)
WO 1996-NL409 19961021
None PCT 102(e) date

DT Utility
FS APPLICATION
LREP YOUNG & THOMPSON, 745 SOUTH 23RD STREET 2ND FLOOR, ARLINGTON, VA, 22202
CLMN Number of Claims: 39
ECL Exemplary Claim: 1
DRWN 9 Drawing Page(s)
LN.CNT 1329
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A protein obtainable from a non pathgenic microorgansim, said protein having mucosa binding promoting activity and a molecular weight of 20-40 kD is disclosed. Application of such a protein or a peptide derived therefrom in a method of screening non pathogenic microorganisms for a microorganism capable of specifically binding mucosa, said method comprising detection in a manner known per se of the presence of a particular protein on or in a microorganism or in a culture of microorganisms, said particular protein being the already defined protein. Kits suitable for such a screening method are also disclosed. Use of a component selected from the group of components comprising a protein or peptide as defined; an **expression** vector comprising nucleic acid encoding such protein or peptide; a recombinant microorganism or a part of said microorganism **expressing** such protein or peptide, said part **expressing** mucosa binding promoting activity; a non pathogenic microorganism capable of **expressing** such protein or peptide or a part of said microorganism, said part **expressing** mucosa binding promoting activity as pharmaceutically active component in a pharmaceutical composition for prophylaxis and/or treatment of disease or illness associated with a mucosa colonizing pathogenic microorganism. Use of such components as food additive and compositions comprising such components are described.

L25 ANSWER 37 OF 48 USPATFULL
AN 2001:82520 USPATFULL
TI Acid-inducible promoters for gene **expression**
IN Kullen, Martin J., Raleigh, NC, United States
Klaenhammer, Todd R., Raleigh, NC, United States
PA North Carolina State University, Raleigh, NC, United States (U.S. corporation)
PI US 6242194 B1 20010605
AI US 2000-637968 20000811 (9)
RLI Continuation of Ser. No. US 1999-336861, filed on 21 Jun 1999, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: LeGuyader, John L.; Assistant Examiner: Zara, Jane
LREP Myers Bigel Sibley & Sajovec
CLMN Number of Claims: 22
ECL Exemplary Claim: 16
DRWN 8 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 951
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An isolated polynucleotide encoding an acid-inducible, or acid-responsive, promoter element includes the F.sub.1 F.sub.0 -ATPase promoter of Lactobacillus acidophilus DNA that hybridizes thereto and

encodes an acid-inducible promoter. Recombinant molecules comprising the promoter operatively associated with a DNA of interest, along with vectors and host cells containing the same, are also disclosed. Methods of upregulating the transcription of a DNA of interest in a host cell with such promoters are also disclosed.

L25 ANSWER 38 OF 48 USPATFULL
AN 2001:78932 USPATFULL
TI Thermophilic polymerase III holoenzyme
IN McHenry, Charles S., Denver, CO, United States
Seville, Mark, Denver, CO, United States
Cull, Millard G., Denver, CO, United States
PA University Technology Corporation, CO, United States (U.S. corporation)
PI US 6238905 B1 20010529
AI US 1997-928213 19970912 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Stole, Einar
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 39
ECL Exemplary Claim: 1
DRWN 29 Drawing Figure(s); 18 Drawing Page(s)
LN.CNT 4725
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to gene and amino acid sequences encoding DNA polymerase III holoenzyme subunits and structural genes from thermophilic organisms. In particular, the present invention provides DNA polymerase III holoenzyme subunits of T. thermophilus. The present invention also provides antibodies and other reagents useful to identify DNA polymerase III molecules.

L25 ANSWER 39 OF 48 USPATFULL
AN 2001:47822 USPATFULL
TI **Expression** and secretion of heterologous polypeptides from caulobacter
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
Nomellini, John F., Richmond, Canada
PA The University of British Columbia, Canada (non-U.S. corporation)
PI US 6210948 B1 20010403
WO 9734000 19970918
AI US 1999-142648 19990330 (9)
WO 1997-CA167 19970310
19990330 PCT 371 date
19990330 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1996-614377, filed on 12 Mar 1996, now patented, Pat. No. US 5976864 Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, now patented, Pat. No. US 5500353 Continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Nashed, Nashaat T.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 1362
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB DNA constructs are provided which code for a chimeric protein in which the C-terminal region corresponds to the extreme C-terminal amino acids of a Caulobacter **S-layer protein**, fused with a heterologous polypeptide. Bacterial cells containing the DNA constructs, or which **express** the DNA constructs and secrete

the resulting protein, are provided. Chimeric proteins including the C-terminal amino acids of a Caulobacter **S-layer protein** are provided, including proteins which include antigenic epitopes of the Infectious Hematopoietic Necrosis Virus.

L25 ANSWER 40 OF 48 USPATFULL
AN 1999:137013 USPATFULL
TI **Expression** and secretion of heterologous polypeptides from caulobacter
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
Nomellini, John F., Richmond, Canada
PA The University of British Columbia, Canada (non-U.S. corporation)
PI US 5976864 19991102
AI US 1996-614377 19960312 (8)
RLI Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, now patented, Pat. No. US 5500353 which is a continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Nashed, Nashaat T.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 14
ECL Exemplary Claim: 2
DRWN 14 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 1609
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB DNA constructs are provided which code for at least the extreme C-terminal amino acids of the rsaA protein of Caulobacter crescentus fused with heterologous polypeptides. Baterial cells containing, or which **express** the DNA constructs and secrete the resulting protein are also provided. Chimeric proteins including the C-terminal amino acids of the rsaA protein are provided, including chimeric proteins comprising antigenic epitopes of the Infectious Hematopoietic Necrosis Virus.

L25 ANSWER 41 OF 48 USPATFULL
AN 1999:24489 USPATFULL
TI **Expression** of surface layer proteins
IN Deblaere, Rolf Y., Waarschoot, Belgium
Desomer, Jan, Drongen, Belgium
Dhaese, Patrick, Drongen, Belgium
PA Solvay (Societe Anonyme), Brussels, Belgium (non-U.S. corporation)
PI US 5874267 19990223
WO 9519371 19950720
AI US 1996-682517 19960917 (8)
WO 1995-EP147 19950113
19960917 PCT 371 date
19960917 PCT 102(e) date
PRAI GB 1994-650 19940114
DT Utility
FS Granted
EXNAM Primary Examiner: Hutzell, Paula K.; Assistant Examiner: Bakalyar, Heather A.
LREP McDermott, Will & Emery
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 41 Drawing Figure(s); 37 Drawing Page(s)
LN.CNT 2742
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A host cell which is provided with a S-layer comprising a fusion polypeptide consisting essentially of:

(a) at least sufficient of a **S-layer protein**

for a S-layer composed thereof to assemble, and

(b) a heterologous polypeptide which is fused to either the carboxy terminus of (a) or the amino terminus of (a) and which is thereby presented on the outer surface of the said cell; can be used as a vaccine, for screening for proteins and antigens and as a support for immobilizing an enzyme, peptide or antigen. A process of **transforming** B. Sphaericus cells comprising electroporation is also provided.

L25 ANSWER 42 OF 48 USPATFULL
AN 1998:85822 USPATFULL
TI Gene and protein applicable to the preparation of vaccines for rickettsia prowazekii and rickettsia typhi and the detection of both
IN Carl, Mitchell, San Diego, CA, United States
Dobson, Michael E., Rockville, MD, United States
Ching, Wei-Mei, Bethesda, MD, United States
Dasch, Gregory A., Wheaton, MD, United States
PA The United States of America as represented by the Secretary of the Navy, Washington, DC, United States (U.S. government)
PI US 5783441 19980721
AI US 1993-169927 19931220 (8)
RLI Continuation-in-part of Ser. No. US 1991-742128, filed on 9 Aug 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Shaver, Jennifer
LREP Spevack, A. David, Garvert, William C.
CLMN Number of Claims: 2
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 928
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB All or part of the DNA sequence of the gene which encodes the **S-layer protein** of R. prowazekii as illustrated in Sequence ID No. 1 as well as a truncated identical piece of this gene in R. typhi as well as the 5' and 3' noncoding regions can be used for vaccination against typhus and spotted fever rickettsial infection or to diagnose the diseases caused by these bacteria. The invention is also accomplished by the deduced amino acid sequence of the **S-layer protein** of R. prowazekii derived from the DNA sequence of the encoding gene. Further, the invention includes the peptide or protein products based on all or parts of this gene.

L25 ANSWER 43 OF 48 USPATFULL
AN 97:59181 USPATFULL
TI Time dependent administration of oligosaccharide glycosides related to blood group determinants having a type I or type II core structure in reducing inflammation in a sensitized mammal arising form exposure to an antigen
IN Ippolito, Robert M., Edmonton, Canada
Haque, Wasimul, Edmonton, Canada
Jiang, Cong, San Diego, CA, United States
Hanna, H. Rizk, Edmonton, Canada
Venot, Andre P., Agoura Hills, CA, United States
Nikrad, Pandurang V., Edmonton, Canada
Kashem, Mohammed A., Thousand Oaks, CA, United States
Smith, Richard, Edmonton, Canada
Srivastava, Om P., Jackson Heights, Canada
PA Alberta Research Council, Alberta, Canada (non-U.S. corporation)
PI US 5646123 19970708
AI US 1995-405785 19950317 (8)
RLI Continuation of Ser. No. US 1993-81214, filed on 25 Jun 1993, now abandoned which is a continuation of Ser. No. US 1992-988518, filed on

10 Dec 1992, now abandoned which is a continuation-in-part of Ser. No. US 1992-895930, filed on 9 Jun 1992, now abandoned which is a continuation-in-part of Ser. No. US 1992-889017, filed on 26 May 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-714161, filed on 10 Jun 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Fonda, Kathleen K.
LREP Burns, Doane, Swecker & Mathis, LLP
CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN 66 Drawing Figure(s); 63 Drawing Page(s)
LN.CNT 6831
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are methods for reducing the degree of antigen induced inflammation in a sensitized mammals. The disclosed methods employ oligosaccharide glycosides related to blood group determinants having a type I or type II core structure wherein the administration of such oligosaccharide glycosides is after initiation of the mammal's immune response but at or prior one-half the period of time required to effect maximal antigen-induced inflammation.

L25 ANSWER 44 OF 48 USPATFULL
AN 96:77808 USPATFULL
TI Methods for the synthesis of monofucosylated oligosaccharides terminating in di-N-acetyllactosaminyl structures
IN Kashem, Mohammed A., Edmonton, Canada
Venot, Andre P., Edmonton, Canada
Smith, Richard, Edmonton, Canada
PA Alberta Research Council, Alberta, Canada (non-U.S. corporation)
PI US 5550155 19960827
AI US 1994-323100 19941014 (8)
RLI Continuation of Ser. No. US 1992-914172, filed on 14 Jul 1992, now patented, Pat. No. US 5374655 which is a continuation-in-part of Ser. No. US 1992-889017, filed on 26 May 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-771259, filed on 2 Oct 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-714161, filed on 10 Jun 1991
DT Utility
FS Granted
EXNAM Primary Examiner: Kight, III, John; Assistant Examiner: Leary, Louise N.
LREP Burns, Doane, Swecker & Mathis
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 1837
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Monofucosylated and monosialyated derivatives of the compound .beta.Gal(1-4).beta.GlcNAc(1-3).beta.Gal(1-4).beta.GlcNAc-OR, where R is hydrogen, a saccharide, an oligosaccharide or an aglycon moiety have been found to be useful in modulating a cell-mediated immune inflammatory response in mammals.

L25 ANSWER 45 OF 48 USPATFULL
AN 94:110797 USPATFULL
TI Methods for the synthesis of monofucosylated oligosaccharides terminating in di-N-acetyllactosaminyl structures
IN Kashem, Mohammed, Edmonton, Canada
Venot, Andre P., Edmonton, Canada
Smith, Richard, Edmonton, Canada
PA Alberta Research Council, Edmonton, Canada (non-U.S. corporation)
PI US 5374655 19941220
AI US 1992-914172 19920714 (7)
RLI Continuation-in-part of Ser. No. US 1992-889017, filed on 26 May 1992

which is a continuation-in-part of Ser. No. US 1991-771259, filed on 2 Oct 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-714161, filed on 10 Jun 1991
PRAI WO 1992-251 19920610
DT Utility
FS Granted
EXNAM Primary Examiner: Russel, Jeffrey E.; Assistant Examiner: Leary, Louise N.
LREP Burns, Doane, Swecker & Mathis
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 2027
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are methods for the preparation of monofucosylated and sialylated derivatives of the compound .beta.Gal(1-4).beta.GlcNAc(1-3).beta.Gal(1-4).beta.GlcNAc-OR. In particular, the methods of this invention provide for a multi-step synthesis wherein selective monofucosylation is accomplished on the 3-hydroxy group on only one of the GlcNAc units found in the .beta.Gal(1-4) .beta.GlcNAc (1-3) .beta.Gal (1-4) .beta.GlcNAc-OR compound. In this step, monofucosylation is achieved by use of the .alpha.(1-3)fucosyltransferase.

L25 ANSWER 46 OF 48 USPATFULL
AN 91:68711 USPATFULL
TI Immunogenic compositions containing ordered carriers
IN Sleytr, Uwe B., Vienna, Austria
Mundt, Wolfgang, Vienna, Austria
Messner, Paul, Vienna, Austria
Smith, Richard H., Edmonton, Canada
Unger, Frank M., Edmonton, Canada
PA Chembiomed, Ltd., Edmonton, Canada (non-U.S. corporation)
PI US 5043158 19910827
AI US 1990-487729 19900302 (7)
RLI Continuation-in-part of Ser. No. US 1988-233674, filed on 18 Aug 1988, now abandoned
PRAI DE 1987-3717897 19870821
CA 1988-575340 19880822
DT Utility
FS Granted
EXNAM Primary Examiner: Russel, Jeffrey E.; Assistant Examiner: Kim, Kay
LREP Irell & Manella
CLMN Number of Claims: 6
ECL Exemplary Claim: 1
DRWN 7 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1000
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Superior pharmaceutical compositions which comprise carriers coupled to epitope-bearing moieties are described. The carriers are **crystalline** or paracrystalline glycoproteins, especially those derived from S-layers of microbial cell walls. These conjugates are capable of eliciting the formation of antibodies as well as a T-cell response.

L25 ANSWER 47 OF 48 USPATFULL
AN 91:52276 USPATFULL
TI Use of structure with membrane having continuous pores
IN Sleytr, Uwe, 10 Parhamerplatz, A-1170 Vienna, Austria
Sara, Margit, 90/2/24 Vorgartenstr, A-1200 Vienna, Austria
PI US 5028335 19910702
AI US 1989-348779 19890508 (7)
RLI Continuation-in-part of Ser. No. US 1988-174127, filed on 28 Mar 1988, now patented, Pat. No. US 4849109, issued on 28 Apr 1989 which is a continuation-in-part of Ser. No. US 1985-795349, filed on 28 Oct 1985,

now patented, Pat. No. US 4752395, issued on 28 Jun 1988
PRAI AT 1984-797 19840309
DT Utility
FS Granted
EXNAM Primary Examiner: Spear, Frank
LREP Bierman and Muserlian
CLMN Number of Claims: 30
ECL Exemplary Claim: 3,20
DRWN 16 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 1039
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Use of novel membrane crystal lattice structures with continuous pores as ultrafiltration membranes, gas separating organs and separating organs for ion exchange processes, structure for immobilizing molecules, and films as enveloping material for substances.

L25 ANSWER 48 OF 48 USPATFULL
AN 89:58419 USPATFULL
TI Use of structure with membrane having continuous pores
IN Sleytr, Uwe, 10 Parhamerplatz, A-1170 Vienna, Austria
Sara, Margit, 90/2/24 Vorgartenstr, A-1200 Vienna, Austria
PI US 4849109 19890718
AI US 1988-174127 19880328 (7)
RLI Continuation-in-part of Ser. No. US 1985-795349, filed on 28 Oct 1985, now patented, Pat. No. US 4752395
PRAI AT 1984-797 19840309
DT Utility
FS Granted
EXNAM Primary Examiner: Jones, W. Gary
LREP Bierman and Muserlian
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 16 Drawing Figure(s); 6 Drawing Page(s)
LN.CNT 954
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Use of novel membrane crystal lattice structures with continuous pores as ultrafiltration membranes, gas separating organs and separating organs for ion exchange processes structure for immobilizing molecules, and films as enveloping material for substances.

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in at least one of the files. Refer to file specific help messages
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L25 ANSWER 1 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2002:236731 BIOSIS

```
DN   PREV200200236731
TI   The surface layer (S-layer) glycoprotein of Geobacillus stearothermophilus
     NRS 2004/3a. Analysis of its glycosylation.
AU   Schaeffer, Christina (1); Wugeditsch, Thomas; Kaehlig, Hanspeter;
     Scheberl, Andrea; Zayni, Sonja; Messner, Paul
CS   (1) Zentrum fuer Ultrastrukturforschung, Ludwig Boltzmann-Institut fuer
     Molekulare Nanotechnologie, Universitaet fuer Bodenkultur Wien,
     Gregor-Mendel-Strasse 33, A-1180, Wien: crs@edv1.boku.ac.at Austria
SO   Journal of Biological Chemistry, (February 22, 2002) Vol. 277, No. 8, pp.
     6230-6239. http://www.jbc.org/. print.
     ISSN: 0021-9258.
DT   Article
LA   English


=> d his

     (FILE 'HOME' ENTERED AT 10:28:46 ON 10 MAY 2002)

     FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS,
     LIFESCI, CAPLUS, USPATFULL, USPAT2' ENTERED AT 10:29:15 ON 10 MAY 2002
                 E LUBITZ WERNER/AU
L1             842 S E1-E3
                 E SLEYTR UWE/AU
L2             235 S E1-E5
                 E SLEYTR U/AU
L3              44 S E3
                 E KUEN BEATRIX/AU
L4              27 S E1-E3
                 E KUEN B/AU
L5              48 S E3
                 E TRUPPE MICHAELA/AU
L6              13 S E1-E4
                 E HOWORKA STEFAN/AU
L7              20 S E3-E5
                 E HOWORKA S/AU
L8              53 S E3-E8
                 E RESCH STEPHANKA/AU
L9               5 S E1-E2
L10             86 S RESCH S/AU
                 E RESCH S/AU
L11             86 S E3
                 E SCHROLL GERHARD/AU
L12             12 S E3
                 E SCHROLL G/AU
L13             43 S E3
                 E SARA MARGIT/AU
L14            150 S E1-E3
                 E SARA M/AU
L15            378 S E3-E4
L16           1634 S L1-L15
L17            258 S L16 AND S LAYER PROTEIN
L18            118 S  L17 AND (MATURE OR COMPLETE OR CRYSTALLINE)
L19             43 DUP REM L18 (75 DUPLICATES REMOVED)
L20             21 S L18 AND (FUSION OR HETEROLOGOUS OR RECOMBINANT)
L21              7 DUP REM L20 (14 DUPLICATES REMOVED)
L22           1102 S S LAYER PROTEIN
L23            304 S L22 AND (MATURE OR CRYSTALLINE OR COMPLETE)
L24            116 DUP REM L23 (188 DUPLICATES REMOVED)
L25             48 S L24 AND (EXPRESS? OR TRANSFORM?)

=> s l25 and (fusion or heterologous)
L26             19 L25 AND (FUSION OR HETEROLOGOUS)
```

=> d bib 1-19

L26 ANSWER 1 OF 19 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:289673 BIOSIS
DN PREV200100289673
TI Analysis of the structure-function relationship of the **S-layer protein** SbsC of Bacillus stearothermophilus ATCC 12980 by producing truncated forms.
AU Jarosch, Marina; Egelseer, Eva M.; Huber, Carina; Moll, Dieter; Mattanovich, Diethard; Sleytr, Uwe B.; Sara, Margit (1)
CS (1) Centre for Ultrastructure Research, Ludwig Boltzmann-Institute for Molecular Nanotechnology, University of Agricultural Sciences, 1180, Vienna: sara@edv1.boku.ac.at Austria
SO Microbiology (Reading), (May, 2001) Vol. 147, No. 5, pp. 1353-1363. print.
ISSN: 1350-0872.
DT Article
LA English
SL English

L26 ANSWER 2 OF 19 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:113721 BIOSIS
DN PREV200100113721
TI The **S-layer protein** of Lactobacillus acidophilus ATCC 4356: Identification and characterisation of domains responsible for S-protein assembly and cell wall binding.
AU Smit, Egbert; Oling, Frank; Demel, Rudy; Martinez, Beatriz; Pouwels, Peter H. (1)
CS (1) Department of Applied Microbiology and Gene Technology, TNO Nutrition and Food Research Institute, Utrechtseweg 48, 3700 AJ, Zeist: Pouwels@voeding.tno.nl Netherlands
SO Journal of Molecular Biology, (12 January, 2001) Vol. 305, No. 2, pp. 245-257. print.
ISSN: 0022-2836.
DT Article
LA English
SL English

L26 ANSWER 3 OF 19 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:12819 BIOSIS
DN PREV200100012819
TI Characterization of the collagen-binding **S-layer protein** CbsA of Lactobacillus crispatus.
AU Sillanpaa, Jouko; Martinez, Beatriz; Antikainen, Jenni; Toba, Takahiro; Kalkkinen, Nisse; Tankka, Sanna; Lounatmaa, Kari; Keranen, Jaakko; Hook, Magnus; Westerlund-Wikstrom, Benita; Pouwels, Peter H.; Korhonen, Timo K. (1)
CS (1) Division of General Microbiology, Department of Biosciences, University of Helsinki, FIN 00014, Helsinki: timo.korhonen@helsinki.fi Finland
SO Journal of Bacteriology, (November, 2000) Vol. 182, No. 22, pp. 6440-6450. print.
ISSN: 0021-9193.
DT Article
LA English
SL English

L26 ANSWER 4 OF 19 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1996:219135 BIOSIS
DN PREV199698775264
TI **Expression** and purification of the **crystalline** surface layer protein of Rickettsia typhi.
AU Hahn, Myong-Joon (1); Chang, Woo-Hyun
CS (1) Dep. Microbiol., Coll. Med., Kon-Kuk Univ., Danwol-Dong, Choongju 380-701 South Korea

SO Microbiology and Immunology, (1996) Vol. 40, No. 3, pp. 233-236.
ISSN: 0385-5600.
DT Article
LA English

L26 ANSWER 5 OF 19 MEDLINE
AN 1998195731 MEDLINE
DN 98195731 PubMed ID: 9534241
TI The S-layer gene of Lactobacillus helveticus CNRZ 892: cloning, sequence and **heterologous expression**.
AU Callegari M L; Riboli B; Sanders J W; Cocconcelli P S; Kok J; Venema G; Morelli L
CS Istituto di Microbiologia, Piacenza, Italy.. mcallega@cr.unicatt.it
SO MICROBIOLOGY, (1998 Mar) 144 (Pt 3) 719-26.
Journal code: BXW; 9430468. ISSN: 1350-0872.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AJ001931; GENBANK-X91199
EM 199805
ED Entered STN: 19980520
Last Updated on STN: 20000303
Entered Medline: 19980512

L26 ANSWER 6 OF 19 MEDLINE
AN 97422863 MEDLINE
DN 97422863 PubMed ID: 9276928
TI Molecular biology of S-layers.
AU Bahl H; Scholz H; Bayan N; Chami M; Leblon G; Gulik-Krzywicki T; Shechter E; Fouet A; Mesnage S; Tosi-Couture E; Gounon P; Mock M; Conway de Macario E; Macario A J; Fernandez-Herrero L A; Olabarria G; Berenguer J; Blaser M J; Kuen B; Lubitz W; Sara M; Pouwels P H; Kolen C P; Boot H J; Resch S
CS Universitat Rostock, Germany.
NC RO1 24145
SO FEMS MICROBIOLOGY REVIEWS, (1997 Jun) 20 (1-2) 47-98. Ref: 197
Journal code: AO4; 8902526. ISSN: 0168-6445.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LA English
FS Priority Journals
OS GENBANK-U38842; GENBANK-X91199; GENBANK-X92752
EM 199710
ED Entered STN: 19971013
Last Updated on STN: 19971013
Entered Medline: 19971001

L26 ANSWER 7 OF 19 WPIDS (C) 2002 THOMSON DERWENT
AN 1996-171046 [17] WPIDS
CR 1994-066249 [09]
DNC C1996-053926
TI **Expression** and presentation of polypeptide **heterologous** to Caulobacter **S-layer protein** to bacterium's environment - by cloning in-frame into Caulobacter rsaA gene, and **expressing** as **fusion** prod. with **S-layer protein**.
DC B04 C06 D16 F09
IN BINGLE, W H; SMIT, J
PA (UYBR-N) UNIV BRITISH COLUMBIA
CYC 1
PI US 5500353 A 19960319 (199617)* 22p
ADT US 5500353 A CIP of US 1992-895367 19920609, US 1994-194290 19940209

PRAI US 1994-194290 19940209; US 1992-895367 19920609

L26 ANSWER 8 OF 19 WPIDS (C) 2002 THOMSON DERWENT
AN 1994-066249 [09] WPIDS
CR 1996-171046 [17]
DNC C1994-029749
TI Prodn. of **heterologous** polypeptides in bacteria, partic. Caulobacter - by **expression** of a **fusion** prod. of the polypeptide sequence and a bacterial **S-layer protein** gene.
DC B04 C06 D15 D16
IN BINGLE, W H; SMIT, J
PA (UYBR-N) UNIV BRITISH COLUMBIA
CYC 1
PI CA 2090549 A 19931210 (199409)* 27p
ADT CA 2090549 A CA 1993-2090549 19930226
PRAI US 1992-895367 19920609

L26 ANSWER 9 OF 19 LIFESCI COPYRIGHT 2002 CSA
AN 1998:14212 LIFESCI
TI IV. Molecular biology of S-layers
AU Bahl, H.; Scholz, H.; Bayan, N.*; Chami, M.; Leblon, G.; Gulik-Krzywicki, T.; Shechter, E.; Fouet, A.; Mesnage, S.; Tosi-Couture, E.; Gounon, P.; Mock, M.; De Macario, E.C.; Macario, A.J.L.; Fernandez-Herrero, L.A.; et al.
CS Lab. des Biomembranes, URA 1116 CNRS, Univ. de Paris-Sud, F-91405 Orsay, France
SO pp. 47-98.
Meeting Info.: International workshop structure, biochemistry, molecular biology and applications of microbial S-layers. Rothenburg o.d. Tauber, Germany. Sep 1996.
DT Book
TC Conference; General Review
FS J
LA English
SL English

L26 ANSWER 10 OF 19 USPATFULL
AN 2002:92298 USPATFULL
TI **EXPRESSION** OF SURFACE LAYER PROTEINS
IN DEBLAERE, ROLF Y., WAARSCHOOT, BELGIUM
DESOMER, JAN, DRONGEN, BELGIUM
DHAESE, PATRICK, DRONGEN, BELGIUM
PA MCDERMOTT, WILL, EMERY (non-U.S. corporation)
PI US 2002048816 A1 20020425
AI US 1998-137531 A1 19980821 (9)
RLI Division of Ser. No. US 1996-682517, filed on 17 Sep 1996, GRANTED, Pat. No. US 5874267
PRAI WO 1995-EP147 19950113
GB 1994-650 19940114
DT Utility
FS APPLICATION
LREP MCDERMOTT WILL & EMERY, 600 13TH STREET, N.W., WASHINGTON, DC, 20005-3096
CLMN Number of Claims: 56
ECL Exemplary Claim: 1
DRWN 36 Drawing Page(s)
LN.CNT 3325

L26 ANSWER 11 OF 19 USPATFULL
AN 2002:48024 USPATFULL
TI NOVEL VACCINES AND PHARMACEUTICAL COMPOSITIONS USING MEMBRANE VESICLES OF MICROORGANISMS, AND METHODS FOR PREPARING SAME
IN KADURUGAMUWA, JAGATH L., GUELPH, CANADA

BEVERIDGE, TERRY J., ELORA, CANADA
PI US 2002028215 A1 20020307
AI US 1999-370860 A1 19990809 (9)
DT Utility
FS APPLICATION
LREP DOUGLAS P MUELLER, MERCHANT & GOULD PC, 3100 NORWEST CENTER, 90 SOUTH SEVENTH STREET, MINNEAPOLIS, MN, 55402
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN 35 Drawing Page(s)
LN.CNT 2647
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L26 ANSWER 12 OF 19 USPATFULL
AN 2002:16912 USPATFULL
TI **EXPRESSION** AND SECRETION OF **HETEROLOGOUS** POLYPEPTIDES FROM CAULOBACTER
IN SMIT, JOHN, RICHMOND, CANADA
BINGLE, WADE H., VANCOUVER, CANADA
NOMELLINI, JOHN F., RICHMOND, CANADA
PI US 2002009792 A1 20020124
AI US 1999-379931 A1 19990824 (9)
RLI Continuation of Ser. No. US 1996-614377, filed on 12 Mar 1996, GRANTED, Pat. No. US 5976864 Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, GRANTED, Pat. No. US 5500353 Continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, ABANDONED
DT Utility
FS APPLICATION
LREP Y ROCKY TSAO, FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 021102804
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 11 Drawing Page(s)
LN.CNT 1124
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L26 ANSWER 13 OF 19 USPATFULL
AN 2001:144913 USPATFULL
TI NOVEL ADHERENCE FACTORS OF NON PATHOGENIC MICROORGANISMS AND APPLICATIONS THEREOF FOR SCREENING MICROORGANISMS FOR SPECIFIC PROBIOTIC PROPERTIES; NOVEL PHARMACEUTICAL COMPOSITIONS AND FOOD ADDITIVES COMPRISING SUCH MICROORGANISMS AND ADHERENCE FACTORS
IN LEER, ROBERT JAN, VOORBURG, Netherlands
POUWELS, PIETER HENDRIK, RIJSWIJK, Netherlands
CONWAY, PATRICIA LYNNE, LE PEROUSE NSW, Australia
PI US 2001018048 A1 20010830
AI US 1998-51755 A1 19980930 (9)
WO 1996-NL409 19961021
None PCT 102(e) date
DT Utility
FS APPLICATION
LREP YOUNG & THOMPSON, 745 SOUTH 23RD STREET 2ND FLOOR, ARLINGTON, VA, 22202
CLMN Number of Claims: 39
ECL Exemplary Claim: 1
DRWN 9 Drawing Page(s)
LN.CNT 1329
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L26 ANSWER 14 OF 19 USPATFULL
AN 2001:82520 USPATFULL
TI Acid-inducible promoters for gene **expression**
IN Kullen, Martin J., Raleigh, NC, United States
Klaenhammer, Todd R., Raleigh, NC, United States
PA North Carolina State University, Raleigh, NC, United States (U.S.

corporation)
PI US 6242194 B1 20010605
AI US 2000-637968 20000811 (9)
RLI Continuation of Ser. No. US 1999-336861, filed on 21 Jun 1999, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: LeGuyader, John L.; Assistant Examiner: Zara, Jane
LREP Myers Bigel Sibley & Sajovec
CLMN Number of Claims: 22
ECL Exemplary Claim: 16
DRWN 8 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 951
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L26 ANSWER 15 OF 19 USPATFULL
AN 2001:78932 USPATFULL
TI Thermophilic polymerase III holoenzyme
IN McHenry, Charles S., Denver, CO, United States
Seville, Mark, Denver, CO, United States
Cull, Millard G., Denver, CO, United States
PA University Technology Corporation, CO, United States (U.S. corporation)
PI US 6238905 B1 20010529
AI US 1997-928213 19970912 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Stole, Einar
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 39
ECL Exemplary Claim: 1
DRWN 29 Drawing Figure(s); 18 Drawing Page(s)
LN.CNT 4725
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L26 ANSWER 16 OF 19 USPATFULL
AN 2001:47822 USPATFULL
TI **Expression** and secretion of **heterologous** polypeptides from caulobacter
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
Nomellini, John F., Richmond, Canada
PA The University of British Columbia, Canada (non-U.S. corporation)
PI US 6210948 B1 20010403
WO 9734000 19970918
AI US 1999-142648 19990330 (9)
WO 1997-CA167 19970310
19990330 PCT 371 date
19990330 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1996-614377, filed on 12 Mar 1996, now patented, Pat. No. US 5976864 Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, now patented, Pat. No. US 5500353 Continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Nashed, Nashaat T.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 1362
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L26 ANSWER 17 OF 19 USPATFULL

AN 1999:137013 USPATFULL
TI **Expression** and secretion of **heterologous** polypeptides from caulobacter
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
Nomellini, John F., Richmond, Canada
PA The University of British Columbia, Canada (non-U.S. corporation)
PI US 5976864 19991102
AI US 1996-614377 19960312 (8)
RLI Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, now patented, Pat. No. US 5500353 which is a continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Nashed, Nashaat T.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 14
ECL Exemplary Claim: 2
DRWN 14 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 1609
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L26 ANSWER 18 OF 19 USPATFULL
AN 1999:24489 USPATFULL
TI **Expression** of surface layer proteins
IN Deblaere, Rolf Y., Waarschoot, Belgium
Desomer, Jan, Drongen, Belgium
Dhaese, Patrick, Drongen, Belgium
PA Solvay (Societe Anonyme), Brussels, Belgium (non-U.S. corporation)
PI US 5874267 19990223
WO 9519371 19950720
AI US 1996-682517 19960917 (8)
WO 1995-EP147 19950113
19960917 PCT 371 date
19960917 PCT 102(e) date
PRAI GB 1994-650 19940114
DT Utility
FS Granted
EXNAM Primary Examiner: Hutzell, Paula K.; Assistant Examiner: Bakalyar, Heather A.
LREP McDermott, Will & Emery
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 41 Drawing Figure(s); 37 Drawing Page(s)
LN.CNT 2742
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L26 ANSWER 19 OF 19 USPATFULL
AN 1998:85822 USPATFULL
TI Gene and protein applicable to the preparation of vaccines for rickettsia prowazekii and rickettsia typhi and the detection of both
IN Carl, Mitchell, San Diego, CA, United States
Dobson, Michael E., Rockville, MD, United States
Ching, Wei-Mei, Bethesda, MD, United States
Dasch, Gregory A., Wheaton, MD, United States
PA The United States of America as represented by the Secretary of the Navy, Washington, DC, United States (U.S. government)
PI US 5783441 19980721
AI US 1993-169927 19931220 (8)
RLI Continuation-in-part of Ser. No. US 1991-742128, filed on 9 Aug 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Shaver, Jennifer

LREP Spevack, A. David, Garvert, William C.
CLMN Number of Claims: 2
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 928
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d clm 18 19

L26 ANSWER 18 OF 19 USPATFULL
CLM What is claimed is:
1. A process of **transforming** B. sphaericus P-1 cells with DNA, which process comprises harvesting B. sphaericus P-1 cells at the late stationary growth phase, mixing the harvested cells with the DNA and effecting electroporation to cause entry of the DNA into the said cells.

L26 ANSWER 19 OF 19 USPATFULL
CLM What is claimed is:
1. A plasmid consisting of a recombinant DNA insert having the nucleotide sequence of Sequence ID No. 1 (or FIG. 4) which encode the surface layer protein of R. prowazekii in a host selected from the group consisting of bacteria, viruses or fungi.

2. The plasmid of claim 1 wherein the host is selected from the group consisting of Escherichia coli, attenuated strains of Salmonella typhi, Bacillus Camille - Guerin (BCG), vaccinia virus, baculovirus **expression** vectors, and yeast.